INCORPORATING HOST-PLANT RESISTANCE INTO SOYBEAN APHID INTEGRATED PEST MANAGEMENT

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Table of Contents

List of Tables

List of Figures

Chapter I: Literature review

7.2 Introduction to soybean aphid

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), originally from Asia, is an invasive pest of soybean in North America. It was first discovered in the United States in Wisconsin in 2000 and was quickly found in most of the Upper Midwest region of the United States within the following year (Venette and Ragsdale 2004; Ragsdale et al. 2011). Prior to the detection of soybean aphid, soybean in the Upper Midwest did not have major insect pressure. In 2000, less than 0.1% of soybean fields in the Upper Midwest were treated with insecticides. Within the next ten years, insecticide use in soybean increased 130-fold in response to soybean aphid and production costs increased by US$16 to $33 per ha (Ragsdale et al. 2007, 2011). When soybean aphid outbreaks have occurred, up to 57% of soybean acres in some states have been treated with foliar insecticides (Ragsdale et al. 2011). Soybean aphid injury to soybean occurs by direct removal of photosynthate, affecting photosynthesis, vectoring plant viruses, honeydew secretion that promotes sooty mold growth, and facilitating population growth of soybean cyst nematode (Macedo et al. 2003; Beckendorf et al. 2008; Ragsdale et al. 2011; Tilmon et al. 2011; McCarville et al. 2014a). These factors can result in up to 40% yield loss (Ragsdale et al. 2007). Control costs and yield losses due to soybean aphid amount to US$ 2.4 to 4.9 billion per year (Song et al. 2006; Kim et al. 2008a).

Soybean aphid has a heteroecious holocyclic life cycle involving primary and secondary host plants, as well as alternation between asexual reproduction during most of the growing season and sexual reproduction in the fall (Ragsdale et al. 2004). Soybean

aphid overwinters as eggs underneath leaf buds of buckthorn, primarily *Rhamnus cathartica* (i.e., primary host), which is also invasive in North America (Heimpel et al. 2010). In late spring, eggs hatch to produce wingless female nymphs, and after approximately three generations on buckthorn, winged morphs are produced that migrate to soybean. Both winged and wingless morphs occur throughout the growing season. Due in part to parthenogenic reproduction, soybean aphid populations can quickly increase to damaging levels under ideal temperature conditions that can result in population size doubling approximately every three days (McCornack et al. 2004). In the fall as temperatures and photoperiod decrease, female and male winged morphs are produced. Females migrate to buckthorn that then produce nymphs eventually capable of sexual reproduction when males arrive from soybean (Ragsdale et al. 2004).

7.2 Integrated pest management

Integrated pest management (IPM) is a decision-making process that uses an understanding of the pest's ecology to reduce the likelihood of pest populations reaching damaging levels and monitoring pest populations to decide if additional action is justified (Radcliffe et al. 2009; Pedigo and Rice 2009). One of the main aspects of IPM is predicting whether a pest is going to cause sufficient yield loss to justify costs of control (e.g, insecticide application). This break-even point is quantified as the economic injury level (EIL) or the pest population density

$$EIL = \frac{C}{VIDK}$$

where *C* is the cost of control, *V* is the value of the crop (e.g., per bushel), *I* is crop injury per pest, and *D* is yield loss per unit of injury, and *K* is the proportion reduction in injury

due to the control tactic (Mitchell and Hutchison 2009). A lower population density than the EIL, the economic threshold (ET), is density where treatment should be initiated to prevent a population from reaching the EIL (Stern et al. 1959; Gray et al. 2009).

IPM also relies on other tactics to prevent pest populations from reaching the EIL before needing to consider insecticide treatment. Biological control utilizes natural enemies such as predators, parasitoids, and pathogens to reduce population growth (Naranjo et al. 2015). Cultural practices such as crop rotation and tilling practices can alter pest abundance, especially for soil dwelling insects (Young et al. 1994). Host-plant resistance is another cornerstone of IPM that can affect both pest numbers and plants' ability to manage insect-induce stresses (Smith 2005).

1.2 Host-plant resistance

Host-plant resistance is a heritable trait that ultimately reduces yield loss caused by an insect (Painter 1951; Smith 2005). Types of host-plant resistance typically fall under one of three categories: antibiosis, antixenosis, and tolerance (Wiseman 1985; Smith 2005). Antibiosis affects pest biology, such as developmental time, survival or fecundity and is detected using no-choice assays where insects are confined to an individual plant to measure population growth (Smith 2005). Antixenosis acts through behavioral avoidance of a plant by reduced feeding, oviposition, or host-preference and is found with choice-tests where insects can freely choose to colonize preferable lines and avoid less preferable lines (Smith 2005). Tolerance does not affect the pest, but instead is the ability of the plant to compensate for damage caused by the pest at otherwise damaging population levels (i.e. reduced yield loss) and is detected in a plant line by

documenting little to no yield decrease between uninfested plants and plants infested with normally damaging pest levels (Smith 2005).

Antibiosis has multiple underlying mechanisms. Most commonly, a plant will utilize phytochemicals toxic to a feeding insect such as terpenes, alkaloids, and various organic compounds (Agrawal et al. 1999). In maize, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is produced in response to mechanical damage to maize leaves (Wahlroos and Virtanen 1959) and can act as a digestive toxin in insects across different feeding guilds such as aphids, lepidopterans, and beetles (Smith 2005). Consuming the chemical can interfere with detoxification enzymes and act similarly to insecticides by inhibiting 4aculate4n44esterase in *Ostrinia* species (Feng et al. 1992). Maysin, a glycoside found in maize silks, also slows development and causes mortality of *Helicoverpa zea,* or corn earworm, larvae feeding on the silks (Waiss et al. 1979). Growth inhibitors can also affect the digestive tract to delay development, or reduce nutrient content due to compounds making nutrients in leaf tissue no longer bioavailable (Faeth and Bultman 1986). Antibiosis can also occur due to structures on the surface of the plant. Trichomes can cause mortality both by physically impaling soft bodied insects causing desiccation (Pillemer and Tingey 1976) and exuding toxic or adhesive substances (Shade et al. 1979).

Antixenosis primarily affects behavior when insects have a choice of different plants to colonize. Visual cues such as green color in barley can make a variety less attractive to *Rhopalosiphum maidis*, the corn leaf aphid, than yellow colored leaves (Moharramipour et al 1997). Plant volatiles are also used as olfactory cues for host suitability that can act as attracts, repellants, or antifeedants (Visser 1986). Antixenosis

can also occur when a plant does not produce an attractant, but another susceptible plant does, such as glucosinolates produced by cabbage that are attractive to *Delia floralis*, the turnip root fly (Hopkins et al. 1997). In addition, alfalfa with dense trichomes tends to be resistant to *Empoasca fabae*, the potato leaf hopper, as the trichomes interfere with the ability to feed and oviposit (Shockley et al. 2002). Similarly, a waxy leaf cuticle can deter feeding and act as a barrier (Stoner 1990).

Tolerance is distinct from antibiosis and antixenosis in that this category of resistance does not affect the pest, but instead focuses on how the plant responds to the pest so that damage does not decrease yield (Smith 2005). Increased photosynthesis, growth, branching, stored nutrients, and moving nutrients to deficient areas of the plant are mechanisms plants can use to compensate for insect feeding damage (Strauss and Agrawal 1999). For instance, maize cultivars tolerant to *Diabrotica virgifera virgifera*, or western corn rootworm, have higher root volume than susceptible cultivars (Rogers et al. 1976). In susceptible wheat plants, chlorophyll content is reduced by aphid feeding, but tolerant varieties are able to repair their photosynthetic system by producing more chlorophyll (Heng-Moss et al. 2003; Cao et al. 2015). In barley, damage from *Schizaphis graminum*, or wheat aphid, is caused by the removal of hormonal auxins that regulate plant growth, and tolerant plants appear to be able to bind auxin in the stem tissue to prevent its removal (Maxwell and Painter 1962). Many of the mechanisms related to tolerance include plant hormones that involve many complex traits that are not yet well documented (Smith 2005).

1.3 Soybean aphid IPM

Currently, soybean aphid IPM relies primarily on monitoring aphid densities and threshold-based applications of broad-spectrum foliar insecticides, primarily pyrethroids and organophosphates (Hodgson et al. 2012). Ragsdale et al. (2007) determined the EIL for soybean aphid was 675 aphids per plant with an EC of 250 aphids per plant while aphid populations are continuing to increase (Koch et al. 2016). Prophylactic neonicotinoid seed treatments are also available, but they are unlikely to provide sufficient control for soybean aphid as concentrations of insecticide in the plant decrease to negligible levels before aphid populations begin to build (Krupke et al. 2017). The adoption of scouting and use of the economic threshold for soybean aphid management is estimated to have produced an economic net benefit of $1.3 billion from 2003 to 2017 (Johnson et al. 2009; Song and Swinton 2009). Current reliance on primarily two insecticide groups is not sustainable due to the high likelihood of insecticide resistance occurring (Pedigo and Rice 2009). Insecticide resistance has been documented in China to both organophosphates and pyrethroids (Wang et al. 2011, 2012; Xi et al. 2015).

Other methods of suppressing soybean aphid populations are also used as part of soybean aphid IPM. When soybean aphid was introduced to North America, it escaped many of the natural enemies it encountered in Asia, but generalist predators in North America have been found feeding on soybean aphid (Ragsdale et al. 2011). *Harmonia axyridis*, the multi-colored Asian lady beetle, is typically the most commonly found predator of soybean aphid and can play a large role in preventing or suppressing large aphid outbreaks (Koch and Costamanga 2017). *Orius insidiosus*, which can reach high levels of abundance early in the growing season, also plays a role in soybean aphid population dynamics (Rutledge et al. 2004). Parasitoids already present in North America

were found parasitizing soybean aphids (Kaiser et al. 2015). In addition, parasitoids from the native range of soybean aphid, such as *Aphelinus certus*, have also become established in Midwest states (Kaser 2016). Soybean aphid can also be attacked by multiple entomopathogens (Koch et al. 2010). However, pesticide use and biological control are not always compatible (reviewed by Desneux et al. 2007). Broad-spectrum insecticides such as pyrethroids can be highly toxic to multiple species of soybean aphid predators (Pezzini et al. 2015, Tran et al. 2016). *A. certus* is also susceptible to dimethoate (i.e., an organophosphate) and λ-cyhalothrin (i.e., a pyrethroid) (Frewin et al. 2012). Because the insecticides commonly used for soybean aphid can affect its natural enemies, aphid populations can quickly resurge after an insecticide application reduces natural enemy populations. While biological control is usually overall more compatible with host-plant resistance than insecticide use (Smith 2005), genes known to confer resistance to soybean aphid can potentially negatively affect predators and parasitoids of soybean aphid either through direct effects on natural enemies or by reduced aphid availability (Lundgren et al. 2009; Heidel-Baker 2012).

1.4 Soybean aphid host-plant resistance

Host-plant resistance has also begun to be utilized against soybean aphid. Hill et al. (2004) first documented the aphid-resistant soybean varieties Dowling and Jackson. Dowling's resistance was determined to be a single dominant gene that was named *Rag1* (or resistance to *Aphis glycines* gene 1). Further work has identified several genes: *Rag1*, *rag1b*, *rag1c*, *Rag2*, *Rag3*, *rag3*, *rag4*, *Rag5*, and *Rag6* (Hesler 2013; Xiao et al. 2013). Soybean varieties containing *Rag1* or a pyramid of *Rag1* and *Rag2* are commercially available for growers (Wiarda et al. 2012; McCarville et al. 2012; Hesler et al. 2013),

although availability can vary regionally (Hanson et al. 2016a). Each gene confers one or more categories of resistance to soybean aphid (Hesler et al. 2013). *Rag3* and *Rag5* exhibit antixenosis, while the remaining *Rag* genes exhibit primarily antibiosis. *Rag1* is reported to exhibit primarily antibiosis and possibly antixenosis, (Diaz-Montano et al. 2006). The underlying physiological mechanism in the plant and interaction with aphids is largely unknown for most Rag genes, but *Rag1* resistance appears to be associated with phytotoxins and lower concentrations of amino acids in the plant (Li et al. 2008; Chiozza et al. 2010; Bansal et al. 2013).

Named *Rag* genes have been categorized as having antibiosis or antixenosis, but tolerance has also been documented in select cases (Pierson et al. 2010; Prochaska et al. 2013). Tolerance against soybean aphid in KS4202 appears to be associated with increased peroxidase levels (Marchi-Werle et al. 2014). The EIL may also be increased on tolerant varieties due to reduced yield loss per number of insects (i.e., *D* in [eq. 1]), and tolerant varieties may not subject soybean aphid to selection pressure towards virulent biotypes as can antibosis or antixenosis (Marchi-Werle et al. 2017).

Soybean aphid management with host-plant resistance is complicated by the presence of aphid biotypes virulent to aphid-resistant plants (Kim et al. 2008b; Hill et al. 2012). In summary, biotype 1 aphids cannot colonize plants with any known *Rag* genes, biotype 2 aphids can colonize plants with *Rag1*, but not *Rag2*, biotype 3 aphids can colonize plants with *Rag2* (Hill et al. 2012), and biotype 4 aphids can colonize plants with *Rag1* and *Rag2* (Alt and Ryan-Mahmutagic 2013). Biotypes 2, 3, and 4 appear to have a fitness cost when reared on susceptible soybean without *Rag* genes (Varenhorst et al. 2015a). Utilizing these fitness costs, refuges containing susceptible plants, and

pyramided varieties may help reduce the prevalence of virulent biotypes (Varenhorst et al. 2015a). Virulent aphids can also induce susceptibility in plants with a *Rag* gene, which can make normally avirulent aphids able to colonize the plants (Varenhorst et al. 2015b). This effect is potentially due to effector proteins secreted by virulent aphids during feeding (Varenhorst et al. 2015c). The presence of biotype 1 aphids on a susceptible plant can also reduce the fitness cost of biotype 2 and biotype 3 (Varenhorst et al. 2015a). In order to manage multiple biotypes, additional sources of resistance will be needed to manage soybean aphid virulence to aphid-resistant plants (Michel et al. 2011; Hesler et al.2013).

1.5 Incorporating soybean aphid host-plant resistance into current management practices

Integration of a resistance trait into elite varieties can require considerable effort, which may in part explain why it may be difficult for growers to find aphid resistant soybean (Hanson et al. 2016a). Screening studies typically examine large numbers of germplasm lines in growth chamber or greenhouse experiments to find a relatively small proportion lines with soybean aphid resistance (e.g., Hill et al. 2004; Hesler and Dashiell 2007, 2008; Hesler et al. 2007, 2012; Rouf Mian et al. 2008; Bansal et al. 2013). These studies also examine the categories of resistance (e.g., antibiosis or antixenosis) with choice or no-choice assays (e.g., Diaz-Montano et al. 2006).

When resistance is identified, the trait is introgressed into elite high-yielding lines with other desirable traits (e. g., Wiarda et al. 2012). However, many resistance traits are discovered in a broad array of germplasm including landraces, historical cultivars, and varieties from other countries; this germplasm can have significantly different genetics

than an elite cultivar, which can create a challenge for breeding because the resistance line can often have other unwanted traits (Tanksley and McCouch 1997; Feuillet et al. 2008). An initial cross would likely result in few if any F2 plants having both the desired agronomic traits such as yield, plant stature, etc. of the elite cultivar and the resistance trait due to recombination events (Smith 2005). Instead, a backcrossing procedure can be used by crossing the F1 progeny back to the elite parent. Resistant progeny are selected and crossed again with the elite parent over multiple generations until the only major difference between the progeny and the susceptible parent (i.e., near-isogenic lines) is the resistance trait (e.g. Komatsu et al. 2008). Linkage mapping is also often performed on recombinant populations to determine what region of the soybean genome is associated with the resistance trait and if a new resistance gene was found (Kim et al. 2010a, 2010b; Jun et al. 2013; Bhusal et al. 2017).

As aphid-resistant soybean varieties become further available, growers will be using the varieties alongside other control tactics such as insecticides that have been used since soybean aphid's arrival in North America. The addition of resistant plants may help slow down the occurrence of insecticide resistance by decreasing the need for insecticides. However, in cases where aphid populations still reach treatable levels, the two tactics may interact either positively or negatively. This potential for interaction justifies the need to evaluate the current efficacy of available insecticides, and assess whether the insecticide susceptibility of aphids feeding on resistant plants is altered.

The following chapters focus on research identifying soybean aphid resistant germplasm and genetic loci to increase the availability of soybean aphid resistance varieties and to understand how use of current insecticides may interact with host-plant

resistance. Such research will include screening for new sources of aphid resistance, genome-wide association mapping using data from published literature to identify markers and positions of potential new *Rag* genes, determining the efficacy of currently used insecticides from populations across Minnesota, and assessing susceptibility to insecticides on resistant plants.

Chapter II: Sources of soybean aphid resistance in early-maturing soybean germplasm

2.1 Introduction

Soybean, *Glycine max* (L.) Merrill, is an important field crop in the United States with 33,613,594 hectares harvested in 2014 (NASS 2015). Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a non-native and damaging pest of soybean, particularly in north central states (Ragsdale et al. 2004). This pest injures soybean directly by removing photosynthate and affecting photosynthesis (Macedo et al. 2003). Indirect damage is caused by soybean aphids vectoring plant viruses, providing substrate for sooty mold growth by secreting honeydew, and facilitating population growth of soybean cyst nematode (Ragsdale et al. 2011; Tilmon et al. 2011; McCarville et al. 2014a). Soybean aphid populations can quickly grow to damaging levels and cost growers $2.4 billion annually (Song et al. 2006). Currently, management of soybean aphid populations relies primarily on threshold-based applications of broad-spectrum foliar insecticides (Ragsdale et al. 2011, Hodgson et al. 2012). Insecticide application is recommended when aphid populations reach an economic threshold of 250 aphids per plant to prevent populations from growing to an economic injury level of 674 aphids per plant (Ragsdale et al. 2007). Adoption of integrated pest management (IPM) programs (i.e., scouting and thresholds) against soybean aphid is estimated to produce an economic net benefit of $1.3 billion from 2003 to 2017 (Johnson et al. 2009; Song and Swinton 2009). However, dependence on broad-spectrum insecticides may result in development of pest resistance to insecticides, replacement by secondary pests, resurgence of the target pest, or environmental contamination (Pedigo and Rice 2009).

Integration of preventive tactics (e.g., host-plant resistance) with therapeutic tactics (e.g., foliar insecticides), may lead to more sustainable soybean aphid management (Pedigo 1995). Host-plant resistance is a heritable decrease in plant susceptibility to pests (Painter 1951; Smith 2005). Resistance of plants to insect pests can be divided into three categories that may act independently or in conjunction (Smith 2005). More specifically, resistant plants can affect pests through impacts on pest developmental time, survival or fecundity (i.e., antibiosis) or behavioral avoidance such as reduced oviposition or attractiveness to colonizing pests (i.e., antixenosis) (Painter 1951; Li et al. 2004; Smith 2005). In addition, resistant plants can tolerate greater pest populations without experiencing economic damage (i.e., tolerance) (Smith 2005). Host-plant resistance is a management strategy under development for the soybean aphid (Hill et al. 2012; Hesler et al. 2013).

There are multiple examples in the literature where soybean lines were screened for resistance to soybean aphid (e.g., Hill et al. 2004; Hesler and Dashiell 2007, 2008; Hesler et al. 2007, 2012; Rouf Mian et al. 2008; Bansal et al. 2013). However, relatively few have explicitly looked at early-maturing soybean (Mensah et al. 2005; Bhusal et al. 2013, 2014; Liu et al. 2015). Known sources of soybean aphid resistance have most often been categorized as antibiosis or antixenosis or both (Diaz-Montano et al. 2006; Hesler and Dashiell, 2011; Enders et al. 2014), but tolerance has also been documented in select cases (Pierson et al. 2010; Prochaska et al. 2013; Marchi-Werle et al. 2014). Further work has identified several genes (*Rag1*, *rag1b*, *rag1c*, *Rag2*, *Rag3*, *rag3*, *rag4*, *Rag5*) that confer one or more categories of resistance to soybean aphids (reviewed by Hesler et al. 2013). Soybean varieties containing *Rag1* or a pyramid of *Rag1* and *Rag2* are

commercially available for growers (Brace and Fehr 2012; Wiarda et al. 2012; McCarville et al. 2012; Hesler et al. 2013). *Rag1* is reported to exhibit primarily antibiosis and potentially antixenosis, while *Rag2* exhibits antibiosis (Diaz-Montano et al. 2006; Hesler et al. 2013).

Potential management of soybean aphid with host-plant resistance is complicated by the fact that soybean aphid biotypes virulent to aphid-resistant plants continue to be discovered in North America (Kim et al. 2008b; Hill et al. 2012). In summary, biotype 1 aphids cannot colonize plants with *Rag1* or *Rag2* genes, biotype 2 aphids can colonize plants with *Rag1*, but not *Rag2*, biotype 3 aphids can colonize plants with *Rag2* (Hesler et al. 2013), and biotype 4 aphids can colonize plants that possess either *Rag1*, *Rag2*, or both genes (Alt and Ryan-Mahmutagic 2013). Additional sources of resistance will be needed to manage soybean aphid virulence to aphid-resistant plants (Michel et al. 2011; Hesler 2013; Hesler et al. 2013). Furthermore, pyramiding multiple resistance genes increases efficacy against soybean aphid (McCarville et al. 2014b; Chandrasena et al. 2015). These factors underscore the importance of searching for new sources of germplasm with resistance that can be readily bred into existing high-yielding varieties.

Therefore, as part of an effort to search for new sources of antibiosis and antixenosis aphid-resistance for soybean that could be grown in Minnesota and other northern areas (e.g., maturity groups 000 to I), we screened 78 previously unscreened soybean lines. Soybean lines showing putative resistance were further evaluated to categorize resistance (i.e., antibiosis and antixenosis) and examine performance under field conditions.

2.2 Materials and Methods

2.2.1 Aphid rearing

A laboratory strain of biotype 1 soybean aphids was obtained from the University of Illinois to establish a source colony for laboratory experiments. At the University of Minnesota, the aphids were reared on SD01-76R soybean plants. Pots (10 × 10 × 10 cm) were filled with approximately 700 cu. Cm of potting soil (Sunshine MVP, Sun Gro Horticulture Products). Seeds were planted at a depth of 2 cm and a 1-cm layer of sand was added to the top of the soil to minimize fungus gnat infestation (Harris et al. 1996). Pots were placed in environmental growth chambers (1.8 × 0.8 × 1.2 m) at 25ºC, approximately 70% humidity, and a photoperiod of 16:8 (L:D) h. Twice weekly, pots were bottom-watered by adding water to the flat containing the pots rather than direct watering to avoid disturbing the plants and aphids (e.g., Hill et al. 2004). Throughout these experiments, plant growth stages were determined using the scale developed by Fehr and Caviness (1977). Plants were infested at the V3 to V5 growth stage in a separate chamber by placing infested plant clippings amongst base of the plants to allow aphids to move to the new plants. In order to maintain the colony, plants were replaced once they reached approximately R1 with new plants from the aphid-free chamber.

2.2.2 Laboratory screening

Screening for resistance to soybean aphid was performed on 75 plant introductions (Pis) of unknown susceptibility to soybean aphid that were obtained from the USDA Soybean Germplasm Collection, University of Illinois, Urbana, IL and three lines from the University of Minnesota Soybean Breeding Program not yet tested for susceptibility to soybean aphid (Table 2.1). Each PI was confirmed to not have been previously assessed for soybean aphid resistance by searching the ARS-GRIN database

(USDA 2015). Three varieties previously used as susceptible checks (Williams 82, IA3027, and SD01-76R), two resistant varieties containing the *Rag1* gene (IA3027RA1 and LD05-16121) and a resistant variety with *Rag1* and *Rag2* genes (IA3027RA12) were included as susceptible and resistant checks (Table 2.1). Lines beginning with IA3027 are near-isolines, where parent lines with *Rag1* or *Rag2* genes were backcrossed to the susceptible IA3027 to produce IA3027RA1 and IA3027RA12 (Brace and Fehr 2012; Wiarda et al. 2012; McCarville et al. 2014b). Three seeds per line were planted in plastic pots as described for aphid rearing. Each pot was randomly assigned one of the 84 soybean lines. Each pot was then placed in a flat with 9 pots per flat, and flats were placed in a growth chamber. Plants were grown using the potting and environmental conditions described for aphid rearing. Each pot within a flat was touching each adjacent pot, and flats where placed so pots on the edges of each flat touched edge pots in the adjacent flats. At the VC growth stage, pots were thinned to two plants per pot to reduce plant competition. A "No-see-um" mesh cage (Skeeta, Bradenton FL) was placed around the group of flats to prevent aphids from escaping the chamber. Each plant was then infested with 10 mixed-aged wingless aphids from the source colony by placing a piece of aphid-infested leaf in the axil of one unifoliolate leaf per plant. Aphids could move freely between plants throughout the cage during the experiment (Hill et al. 2004). This methodology was chosen for the initial screening because effects of both antibiosis and antixenosis would affect the number of aphids on each plant. At 14 d after infestation (DAI), plants from each pot were cut at soil level, placed in plastic bags and placed in a freezer to later record total aphids per pot. This experiment was conducted as a randomized complete block design with six blocks over time between 1 July 2013 and 15

Jan. 2014. Each block contained a single pot of each of the 84 lines used in the experiment.

The average number of aphids per plant for each pot was calculated. Each pot was considered an experimental unit, and plants within a pot were considered subsamples. Known susceptible and resistant lines were grouped into susceptible and resistant categories, respectively, to compare to each unknown line. Differences in average aphids per pot among soybean lines were analyzed with SAS 9.4 by analysis of variance (PROC GLM) with main effects for soybean line and block (i.e., date) and the two-way interaction (SAS Institute, 2014). Assumptions of normality and homogeneity of variance were examined by visual inspection of residuals and Levene's test, respectively, and data were natural log-transformed to meet the assumption of homogeneity of variance. One-tailed Dunnett's multiple-comparison tests were used to determine which soybean lines had fewer aphids per plant than the grouped known susceptible soybean lines and more aphids per plant than grouped known resistant lines (e.g., Hazard et al. 2014). Lines with significantly fewer aphids than known susceptible lines were considered resistant instead of comparing lines to resistant checks to classify a line as resistant. This was done to minimize failure to detect resistance in lines with moderate resistance. Comparisons were made to known resistant lines to assess the strength of resistance in a line. Soybean lines exhibiting resistance to soybean aphid were advanced for further experiments to categorize resistance (i.e., antibiosis or antixenosis) and field evaluation.

2.2.3 Antibiosis test

A no-choice experiment was used to test for antibiosis in PI 639534A, PI 639537, PI 605765B, PI 507713, and PI 605819C. In this experiment, Williams 82 and IA3027

were used as susceptible checks and IA3027RA1 was used as a resistant check. Plants were grown to the VC growth stage using the planting methods and environmental conditions described for aphid rearing. Plants were removed by clipping in each pot so only one plant remained prior to infestation. Apterous adult aphids were placed in clip cages with two aphids per cage and cages were clipped to the abaxial surface of unifoliolate leaves with one cage per plant (Hesler and Dashiell, 2007; Hesler et al. 2007). Clip cages consisted of clear 2.5-cm diameter plastic tubing cut approximately 1.3 cm long. "No-see-um" mesh (Skeeta, Bradenton FL) was glued to one side of the tubing and 0.5-cm thick ring of foam was glued to the other side of the tubing. The side of the tube with foam was held in contact with the leaf by a metal clip glued to the side of the tubing (Davis et al. 2005). Total aphids were recorded at 1 and 7 DAI. This experiment was conducted as a generalized randomized complete block design with five replications of each treatment in each of three blocks occurring over time (15 replications total) between 6 and 27 June 2014.

Population increase between 1 and 7 DAI was calculated with SAS 9.4 by determining the slope between the aphid counts at the two sampling dates (PROC REG) within each cage (SAS Institute, 2014). Differences in population increase (i.e., slopes) among soybean lines were analyzed using an analysis of variance (PROC GLIMMIX) with main effects for soybean line and block and the two-way interaction (SAS Institute, 2014). Means were separated with Tukey's multiple comparison test. Assumptions of normality and homogeneity of variance were examined by visual inspection of residuals and Levene's test, respectively, and data were natural log-transformed to meet the assumption of homogeneity of variance (SAS Institute, 2014).

2.2.4 Antixenosis test

A choice-test experiment was used to test for antixenosis in PI 639534A, PI 639537, PI 605765B, PI 507713, and PI 605819C. Plants were grown using the potting soil and environmental conditions described for aphid rearing. Circular pots (30-cm diameter × 25-cm height) were used as choice-test arenas. Pots were filled to a height of 19.5 cm with potting soil. The arena consisted of eight positions evenly spaced in a circle (10-cm diameter and 7.85 cm arc-length between positions). Soybean lines were randomly assigned positions in each arena and two seeds of each soybean line were planted at assigned positions. To reduce plant competition, one plant was removed from each position after emergence. When all plants reached the VC growth stage, 250 mixed-aged non-winged aphids were placed on filter paper in the center of each arena (Diaz-Montano et al. 2006; Hesler and Dashiell, 2011). To prevent aphids from moving between pots, No-see-um mesh (Skeeta, Bradenton FL) was suspended from the ceiling of the chamber and placed over each pot. Aphids were allowed to freely colonize plants by walking from the filter paper to the plants. Aphid counts on each plant in each arena were recorded 1 DAI. The 24-h duration was chosen to minimize the confounding effect of aphid reproduction. This experiment was conducted as a completely randomized block design with eight replications (i.e., pots) repeated on three different dates between 5 Mar. and 9 Apr. 2014. Five pots were excluded from the analysis where fewer than 20 aphids had colonized plants in a pot one DAI or a seedling from one of the lines did not emerge. Data were analyzed as an ANOVA with a binary response count (i.e., aphids on a single plant / total aphids on plants in the pot) using logistic regression (PROC GLIMMIX) to compare the proportion of aphids infesting each plant in a pot (Morawo and Fadamiro,

2014). Differences between pots were considered a random effect. Proportions of aphids infesting each line were compared with Tukey's multiple comparison test.

2.2.5 Field evaluation

The top performing soybean lines (PI 639534A, PI 639537, PI 605765B, and PI 507713), a known resistant line with the *Rag1* gene (Gold Country 1114) and two susceptible lines (Gold Country 0943 and SD01-76R) were evaluated under field conditions at the University of Minnesota Research and Outreach Center near Rosemount (44°42'30.6"N 93°06'02.6"W) and the University of Minnesota Agricultural Experiment Station in St. Paul (44°59'34.6"N 93°10'22.1"W) in 2014. The known resistant and susceptible lines have maturities similar to the resistant lines identified in laboratory experiments and were chosen to minimize the potential confounding effect that differences in plant growth stage could have on aphid population growth (Catangui et al. 2009). The other susceptible and resistant checks used in the laboratory experiments were not used because they are in later maturity groups (Table 2.1). Each soybean line was planted in single-row plots 3.05-m long with 2.54-cm planting depth, 2.54-cm spacing between seeds, and 3.05-m spacing between rows. The seven treatments (i.e., soybean lines) were arranged in a randomized complete block design with four replications at each location. The Rosemount location was planted 10 June with a cone planter and the St. Paul location was hand planted on 13 June. Weeds were controlled with pre-emergent herbicide and rototilling. Aphids were allowed to naturally infest plots. Plots at each location were sampled weekly from 9 July to 15 August (i.e., six weeks per location). On each sample date, plants were randomly selected from each plot and winged and non-winged aphids were counted via nondestructive visual whole-plant inspection. Ten plants

per plot were sampled at the beginning of the experiment and five plants per plot were sampled when > 80% of plants were infested (Ragsdale et al. 2007).

Cumulative aphid days (CAD) were calculated for each plot over the duration of the experiment using,

$$\text{CAD} = \sum_{i=1}^{n} [(x_i + x_{i-1})/2] * t_i$$

where *n* is the number of sample dates at the location, *x* is the average number of aphids per plant on sample date *t*, and t_i is days since the previous sample (Hanafi et al. 1989). Differences in CAD among soybean lines were analyzed by analysis of variance (PROC GLIMMIX) with Tukey's mean separation test with main effects for soybean line and location, and the two-way interaction. Replications within a location were considered a random effect. Assumptions of normality and homogeneity of variance were examined by visual inspection of residuals, and data were natural log-transformed to meet the assumption of homogeneity of variance.

2.3 Results

2.3.1 Laboratory screening

Across the soybean lines evaluated, mean aphids per plant at 14 DAI ranged from 42 to 720 (Fig. 2.1). Mean aphids per plant differed significantly among soybean lines [F (79, 24) = 5.62; $p < 0.01$] and experimental blocks [F (5, 24) = 62.09; $p < 0.01$]. However, there was no significant interaction, indicating that differences among soybean lines did not change among experimental blocks [F (393, 24) = 0.61; $p = 0.97$]. PI 639534A, PI 639537, PI 605765B, and PI 507713 had significantly fewer aphids per plant than the known susceptible lines, and mean aphids per plant for PI 639534A did not differ significantly from the known resistant lines (Fig. 2.1).

2.3.2 Antibiosis test

Population increase (i.e., slopes) from 1 to 7 DAI ranged from 1.6 to 13.8 aphids per day. Population increases were significantly different among soybean lines [F (7, 92) = 22.35; $p < 0.01$] and experimental blocks [F (2; 92) = 6.20; $p < 0.01$]. There was no significant interaction between lines and block [F (14; 92) = 1.46; $p = 0.14$], which indicated that differences among soybean lines did not change among experimental blocks. PI 639534A, PI 639537, and PI 605765B had significantly lower population growth than either known susceptible line (i.e., Williams 82 or IA3027), and population growth for PI 639534A and PI 605765B did not differ significantly from the known resistant line (i.e., IA3027RA1) (Fig. 2.2).

2.3.3 Antixenosis test

On average, 32% of the aphids placed in the center of the pot were found on plants 1 DAI (ranging from 8 to 81%). Preference was significantly different across lines [F (7, 128) = 5.21; $p < 0.01$]. There was not a significant interaction between experiment date and line choice [F (16, 128) = 1.11; $p = 0.36$], therefore soybean line was included as the only independent variable in the model. The proportion of aphids infesting PI 507113 was significantly less than both susceptible lines (i.e., Williams 82 and IA3027) (Fig. 2.3). No difference was detected in preference between IA3027 and IA3027RA1, although aphids had higher preference for Williams 82 than IA3027 or IA3027RA1 (Fig. 2.3). Fewer aphids infested PI 639534A and PI 639537 than Williams 82 but not IA3027 (Fig. 2.3).

2.3.4 Field evaluation

On the first sampling date at each site, approximately 10% of plants were infested experiment-wide, and infested plants had approximately 10 or fewer aphids. At peak infestation on the last sampling dates, mean aphid numbers were approximately 250 and 670 aphids per plant in St. Paul and Rosemount, respectively. CAD varied between locations and among soybean lines, ranging from 379 to 8,524 CAD (Fig. 2.4). CAD differed significantly among soybean lines [F (6, 39) = 29.25; $p < 0.01$] and between locations [F (1, 39) = 139.88; $p < 0.01$]. However, there was no significant interaction [F (6, 39) = 0.84; $p = 0.55$], which indicated that differences among soybean lines did not change between locations. PI 605765B and PI 639537 had significantly lower CAD than the known susceptible soybean lines SD01-76R and Gold Country 0943 (Fig. 2.4). CAD for the previously unevaluated lines and the known susceptible lines were all significantly higher than that of the known resistant soybean line (Fig. 2.4).

2.4 Discussion

In this study, four new sources of soybean aphid resistance were found through laboratory screening against biotype-1 soybean aphids: PI 639534A, PI 639537, PI 605765B, and PI 507713 (Fig. 2.1). The category of resistance appears to be antibiosis for PI 639534A, PI 639537, and PI 605765B (Fig. 2.2). Antixenosis was found in PI 507713 and potentially in PI 639534A and PI 639537 (Fig. 2.3). However, under field conditions, only PI 605765B and PI 639537 showed a lower number of CAD than both susceptible varieties (Fig. 2.4). PI 605819C was included in the antibiosis and antixenosis experiments because it appeared to be potentially resistant in early replications of the laboratory screening experiment, but did not have significantly fewer aphids per plant than susceptible lines at the end of that experiment (Fig. 2.1). Antibiosis or antixenosis

could not be detected in PI 605819C in the categorization experiments (Figs. 2.2 and 2.3). PI 639534A, PI 639537, and PI 605765B are maturity group I, while PI 507713 is maturity group 0. We searched the USDA's Germplasm Resources Information Network (GRIN) for currently reported resistant lines to determine maturity groups of known resistant lines (USDA 2015). As of 4 May 2015, the GRIN database listed 35 unique lines as resistant, including none from maturity group I, six from maturity group 0, and seven from maturity group 00 (Fig. 2.5). However, results of some recent publications may not yet be included in the database, such as the maturity group I soybeans described by Bhusal et al. (2014). The new sources of resistance identified in this study should contribute to future breeding of early-maturing soybean varieties with aphid resistance for the upper Midwest.

Soybean lines in which we did not document resistance should not be entirely excluded from further study with biotype 1 aphids or other biotypes. Because most of the soybean lines examined in this study have not been selected for soybean aphid resistance in a breeding program, the frequency of resistance traits could be low within populations. This could have resulted in some lines with low frequencies of resistance traits appearing susceptible in our experiments. This may be the situation with lines such as PI 605819C that appeared to have low mean aphids in the initial screening experiment, but that had high variation in mean aphids per plant (Fig. 2.1). To quantify the frequency of resistance expression within soybean lines, Hesler et al. (2007) used a threshold method in which they chose a threshold density of aphids as an indicator of resistance and examined the proportion of plants within soybean lines expressing resistance relative to that threshold.

However, we chose not to use their method because the threshold value for defining resistance is arbitrarily selected.

In the antibiosis experiments, PI 507713 had significantly fewer aphids than one susceptible line (IA3027), but not the other susceptible line (Williams 82). This indicates variation in susceptibility of known susceptible lines and highlights the importance of including multiple check lines in a screening experiment. Bhusal et al. (2014) also documented variation among susceptible and resistant checks. This difference could indicate either a small degree of antibiosis in PI 507713 that we could not detect when compared against both susceptible lines, or that antibiosis did not occur if antibiosis is to strictly be interpreted as lower population growth than all susceptible lines. Therefore, we could not rule out a lack of antibiosis in PI 507713 for this experiment.

In the antixenosis experiment, only PI 507713 had significantly fewer aphids than the susceptible IA3027 and Williams 82 lines. The amount of aphids on IA3027, a previously assumed susceptible line, was not significantly different from the resistant IA3027RA1; the other susceptible line, Williams 82, had significantly more aphids than either of the Iowa near-isolines. The strength of antixenosis in specific lines can vary between studies as indicated by Hesler and Dashiell (2011). This variability could be due to differences in aphid biotypes (Hesler and Dashiell, 2011) or differences in lines offered as choices, arena design, or other experimental conditions used in Hill et al. (2004) and Hesler and Dashiell (2011). However, our antixenosis experiment exclusively used biotype 1 aphids, so aphid biotype would not explain the lack of difference between isolines. Another potential explanation is that the background genetics of the IA3027 line, prior to the addition of *Rag1* resulting in the IA3027RA1 line, may have already had

some antixenotic trait that results in decreased preference for both Iowa lines compared to Williams 82 (Fig. 2.3). In addition, there is some doubt whether *Rag1* confers antixenosis in addition to antibiosis or if the antixenosis is due to another resistance gene in lines known to also have the *Rag1* gene (Hesler and Dashiell, 2011). Hill et al. (2012) indicate *Rag1* confers primarily antibiosis. Hill et al. (2006a) demonstrated monogenic resistance in Dowling, but their methodology, which is similar to our initial laboratory screening methodology, may be better suited to detecting either antibiosis or antixenosis affecting nymphiposition behavior. However, plant-choice (i.e., colonization) antixenosis is instead measured over 24 or 48 h after infestation (e.g., Diaz-Montano et al. 2006). Because IA3027 appears to have strong plant-choice antixenosis compared to Williams 82, but no difference in antixenosis from IA3027RA1, we consider both the Iowa lines and PI 507713 to be antixenotic. PI 639537 and PI 639534A could also be considered moderately antixenotic when only compared to Williams 82.

Field experiment results differed from the laboratory experiment results. For example, PI 639534A showed high levels of resistance in the initial screening and antibiosis study, but was one of the least resistant lines under field conditions (Figs. 2.1, 1.2, 1.4). Several factors could contribute to the difference in performance between laboratory and field experiments. Biotype 1 aphids were used in the laboratory, but natural aphid infestations occurred in the field experiments. Locally present aphid biotypes in Minnesota have not been well characterized, but some virulence on *Rag1* and *Rag2* has been documented in Wisconsin (Crossley and Hogg, 2015). In addition, differences in environmental conditions (e.g., temperature, soils, water content, etc.) between field and laboratory experiments could affect expression of resistance.

Chirumamilla et al. (2014) found that resistance was relatively stable across 21 to 28°C for some known resistant lines, but soybean aphids on *Rag2* plants (PI 200538) were less virulent at 28°C than 21°C. Other lines can have variable susceptibility depending upon temperature (Richardson 2012). Whalen and Harmon (2015) also demonstrated that overall soybean aphid population growth increases as temperature increases on susceptible plants, but decreases as temperature increases on resistant plants. In addition, Brunner et al. (2014) found an inverse relationship between soybean aphid number and number of root nodules, which can affect the amount of nodule-colonizing bacteria and thereby affect nitrogen availability. Furthermore, our laboratory experiments were conducted with early vegetative growth stages of soybean (i.e., approximately V2-V3), while field screening occurred on plants up to approximately R6. Physiological responses of the plant can vary between vegetative and reproductive stages and are an avenue for further attention in soybean host-plant resistance (Prochaska et al. 2013). Finally, the apparent lack of resistance of PI 507713 to soybean aphids in the field trial (Fig. 2.4) may have been due to the plant-choice antixenosis effect on initial aphid colonization being overcome by later population growth as the line does not appear to have antibiosis (Fig. 2.2). More detailed sampling during aphid colonization would have been required to detect such an antixenotic effect on new colonizing aphids early in the growing season.

Early-maturing lines found in this study that possess antibiosis or antixenosis will be of use to future breeding programs to better manage soybean aphid. As aphids begin to move from buckthorn to soybean in spring, antixenosis that affects colonization could deter aphids from initially colonizing the plants, which may delay when population growth occurs (Hesler and Dashiell, 2011). If plants in a field have an antixenotic trait,

aphids may initially land on plants, but then fly to other plants in the field before deciding to colonize; this time and energy spent searching could be a fitness cost (Hesler and Dashiell, 2011). Antibiosis, especially when wingless aphids are reproducing on soybean, can slow population growth and significantly reduce the cumulative number of aphids on a plant at the end of the growing season (Wiarda et al. 2012). Combining traits from lines with antibiosis and antixenosis, such as PI 605765B and PI 507713, in a breeding program could produce especially resistant varieties that both repel colonizing aphids and slow the population growth of those aphids that do colonize the plants (Hesler and Dashiell, 2011; Hill et al. 2012).

To advance utilization of these sources of resistance in breeding, further studies are needed to determine the genetic basis for resistance and determine if new *Rag* genes are present (e.g., Hill et al. 2006a; 2006b; 2009). Identification of new *Rag* genes and their utilization in pyramids with other *Rag* genes would improve efficacy of host-plant resistance for soybean aphid (McCarville et al. 2014b) and management of virulent aphid biotypes (Hesler et al. 2013; Wenger et al. 2014). Additional research could also investigate plant-choice antixenosis observed in IA3027, a previously assumed susceptible soybean line.

2.5 Acknowledgements

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2.6 Tables

Table 2.1. Soybean lines used in laboratory and field experiments and relative maturity.

Accession #	Relative Maturity	Accession #	Relative Maturity	Accession #	Relative Maturity
PI 548594	000	PI 605819A	0	PI 639544	1
PI 639637	000	PI 605819C †	0	PI 639545B	1
PI 639627	000	PI 612620	0	PI 639550C	1
PI 639629	000	PI 612621	0	PI 639550D	1
PI 567787	000	PI 639630A	0	PI 639553	1
PI 653905	00	PI 639631	0	PI 605750	1
PI 639633B	00	PI 639632A	0	PI 605765A	1
PI 507713	0	PI 592900	0	PI 605765B †‡	1
PI 548500	0	PI 639632B	0	PI 612622A	1
PI 548560	0	MO6-297013	0.7	PI 639560A	1
PI 548615	0	MO2-483059	0.7	PI 639560B	1
PI 631437	0	MO2-483076	0.8	PI 639630B	1
PI 631438	0	Gold Country 0943 ‡	0.9	PI 556779	1
PI 634813	0	PI 540554	1	PI 612622B	1
PI 638510	0	PI 548526	1	PI 613561	1
PI 638511	0	PI 548668	1	PI 614833	1
PI 639527	0	PI 548673	1	PI 639555A	1
PI 639529A	0	PI 548676	1	PI 639555B	1
PI 639529B	0	PI 548681	1	PI 639556A	1
PI 639538	0	PI 548683	1	PI 639632C	1
PI 639542A	0	PI 550740	1	Gold Country 1114 ‡	1.1
PI 639542B	0	PI 556637	1	LD05-16121 †	2
PI 639547	0	PI 556776	1	SD01-76R ‡	2
PI 639549	0	PI 639528A	1	IA3027	3
PI 639550A	0	PI 639531	1	IA3027RA1 †	3
PI 639550B	0	PI 639534A †‡	1	IA3027RA12	3
PI 639552	0	PI 639537 †‡	1	Williams 82	3.9
PI 639554	0	PI 639539A	1		
PI 639636	0	PI 639543	1		

† Soybean lines used in antibiosis and antixenosis testing.

‡ Soybean lines used in field evaluation.

Known aphid-susceptible lines: IA3027, SD01-76R, Williams 82, Gold Country 0943 (Hill et al. 2004; Wiarda et al. 2012; Bansal et al. 2013). Known aphid-resistant lines: IA3027RA1, IA30227RA1+2, LD05-16121, Gold Country 1114 (Heidel-Baker 2012; Wiarda et al. 2012).

2.7 Figures

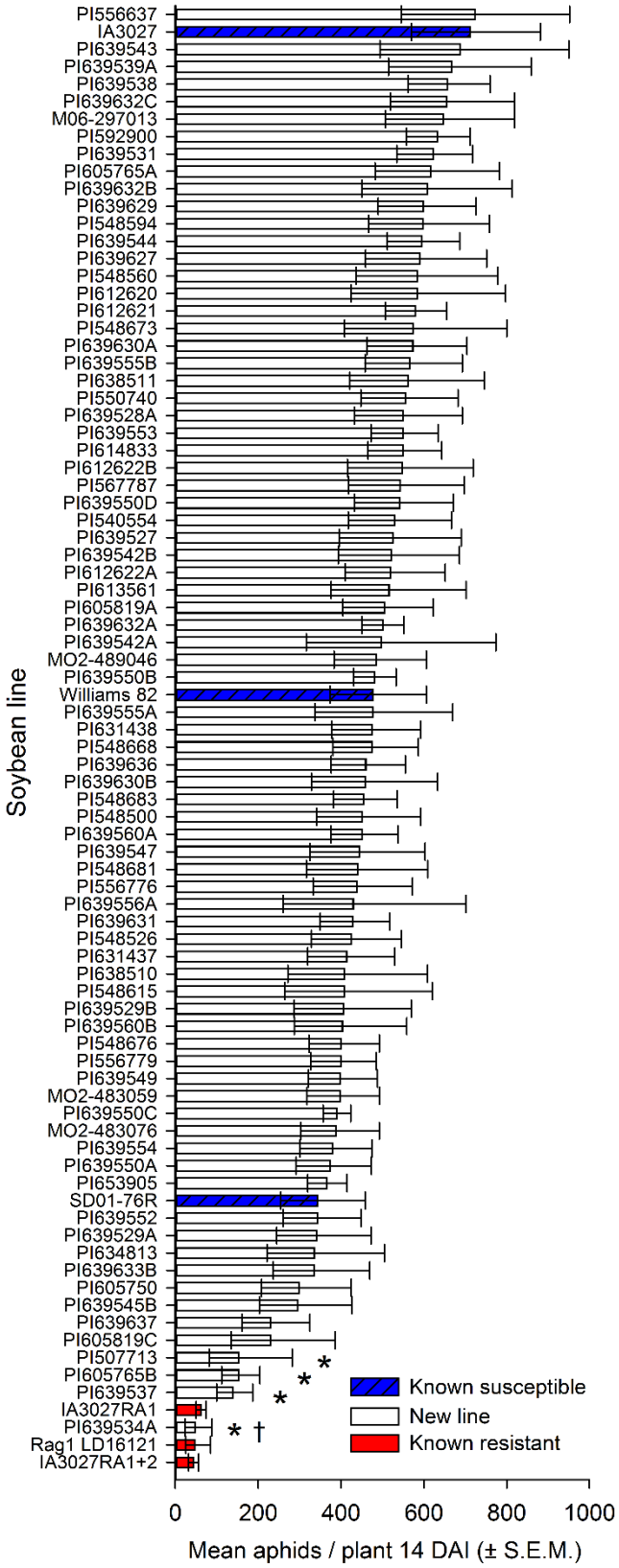

Figure 2.1. Mean number of aphids per plant 14 d after infesting (DAI). Each plant was infested with 10 aphids while allowing aphids to freely move among pots for initial resistance screening. Data are back-transformed from a natural log distribution. Asterisks indicate previously unevaluated soybean lines with significantly fewer mean aphids per plant than the average of the known susceptible lines at $\alpha = 0.05$ (*), 0.01 (**), and 0.001 (***). A dagger (†) indicates a previously unevaluated line that did not have significantly higher mean aphids per plant than known resistant lines at $\alpha = 0.05$.

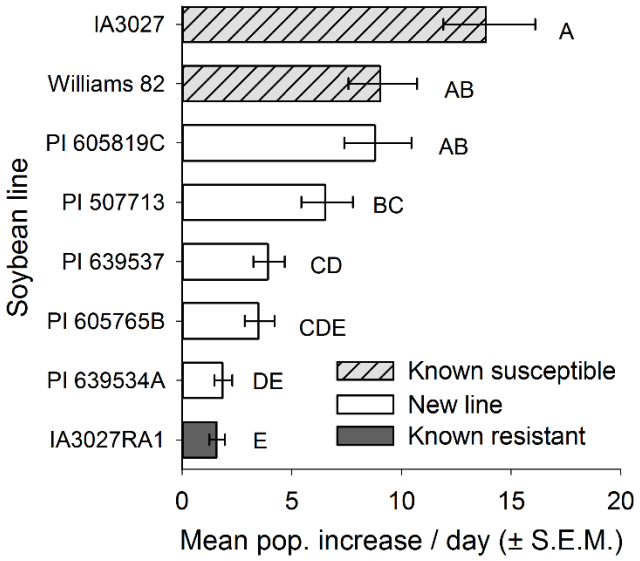

Figure 2.2. Mean population increase (aphids per day) within clip-cages from one to seven days after infestation in a no-choice test for antibiosis. Data are back-transformed from a natural log distribution. Means are not significantly different for soybean lines with the same letter at $\alpha = 0.05$.

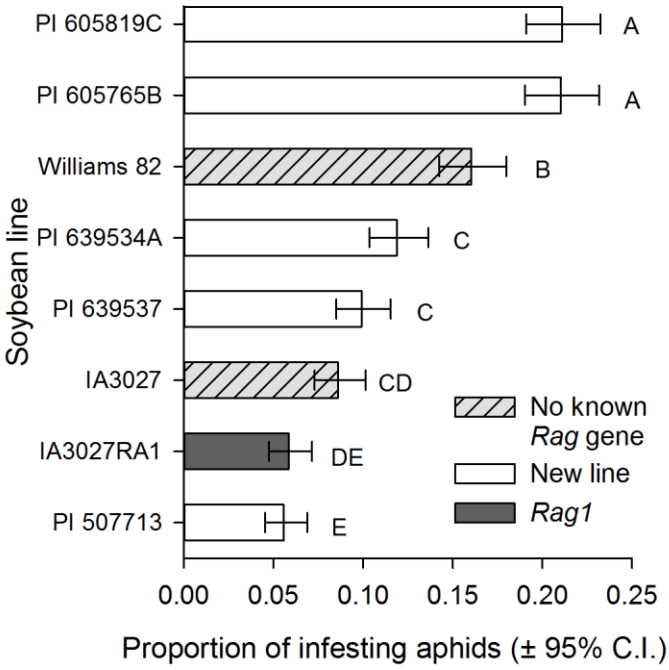

Figure 2.3. Proportion of aphids (± 95% C. I.) colonizing each soybean line in the antixenosis screening 24 h after releasing aphids in center of test arena. Lines without known *Rag* genes were expected to be susceptible, and the line with the *Rag1* gene was expected to be resistant. Logistic regression intercepts (transformed to proportions) are not significantly different for lines with the same letter at $\alpha = 0.05$.

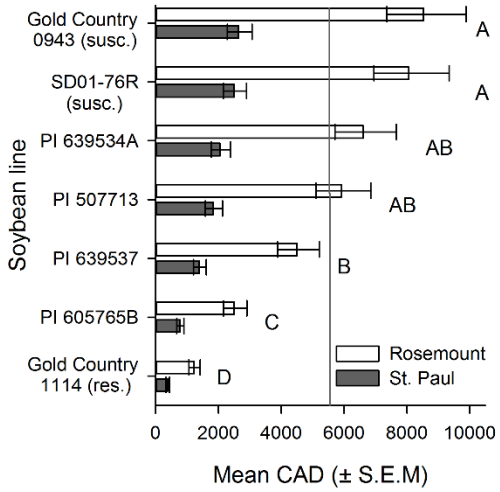

Figure 2.4. Mean cumulative aphid days (CAD) for selected soybean lines at Rosemount and St. Paul, MN between 9 July and 15 Aug 2014 for known susceptible (susc.), known resistant (res.) and resistant lines from lab screening. Data are back-transformed from a natural log distribution. For reference, the gray vertical line represents the economic injury level at 5,563 CAD (Ragsdale et al. 2007). Within locations, means are not significantly different for lines with the same letter $\alpha = 0.05$. Across lines, means are significantly different between locations at $\alpha = 0.05$.

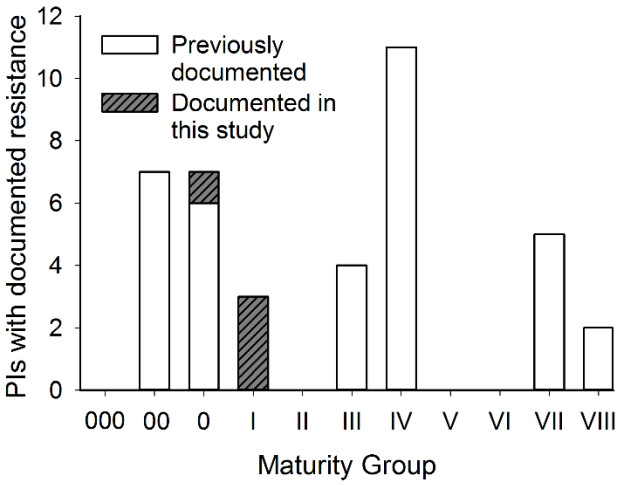

Figure 2.5. Number of soybean aphid resistance sources previously known and found in this study by relative maturity group. Data were obtained from 35 lines listed as resistant in the ARS-GRIN database on 4 May, 2015 (USDA 2015).

Chapter 3: Genome-wide association mapping of host-plant resistance for soybean aphid

3.1 Introduction

Soybean is an important field crop in the United States with 33,482,071 ha harvested in 2016 (USDA-NASS, 2016). Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive and damaging pest of soybean, especially in the upper Midwest of the United States that directly removes photosynthate and affects photosynthesis (Ragsdale et al. 2004; Macedo et al. 2003). In addition, soybean aphid can also vector plant viruses, provide substrate for sooty mold growth by secreting honeydew, and facilitate the population growth of soybean cyst nematode (Ragsdale et al. 2011; Tilmon et al. 2011; McCarville et al. 2014a). Injury caused by soybean aphid infestations can cost growers $2.4 billion annually (Song et al. 2006). Threshold-based applications of broad-spectrum foliar insecticides are the primary tactic used to manage soybean aphid (Ragsdale et al. 2011, Hodgson et al. 2012). However, dependence on broad-spectrum insecticides can result in the development of pest resistance to insecticides, increase outbreaks of secondary pests, resurgence of the target pest, and environmental contamination (Pedigo and Rice, 2009).

Host-plant resistance is a management strategy under development for the soybean aphid to reduce the likelihood of aphid populations causing economically significant yield loss (Hill et al. 2012; Hesler et al. 2013). Host-plant resistance is a heritable decrease in plant susceptibility to pests (Painter 1951; Smith 2005). Resistance of plants to insect pests can be divided into three categories that may act independently or

in conjunction (Smith 2005). More specifically, resistant plants can affect pests through impacts on pest developmental time, survival, or fecundity (i.e., antibiosis) or behavioral avoidance such as reduced oviposition or attractiveness to colonizing pests (i.e., antixenosis) (Painter 1951; Li et al. 2004; Smith 2005). In addition, some resistant plants can tolerate greater pest populations without experiencing economic damage (i.e., tolerance) (Smith 2005). Soybean aphid host-plant resistance was first documented in PI548633 (i.e., Dowling) and PI548657 (i.e., Jackson) (Hill et al. 2004). Known sources of soybean aphid resistance have most often been categorized as involving antibiosis, antixenosis, or both (e.g., Diaz-Montano et al. 2006; Hesler and Dashiell 2011; Enders et al. 2014), but tolerance has also been documented in select cases (Pierson et al. 2010; Prochaska et al. 2013; Marchi-Werle et al. 2014).

Over 3,500 soybean lines have been screened for resistance to soybean aphid with at least 39 lines exhibiting resistance (Cooper et al. 2015; Hanson et al. 2016b). Genetic mapping of these lines has been used to identify several genes (Table 3.1) that confer one or more categories of resistance to soybean aphids (reviewed by Hill et al. 2012; Hesler et al. 2013). Soybean is an inbreeding diploid crop that is subject to limited genetic diversity in elite lines, so elite lines are often outcrossed for beneficial traits (Chung and Signh 2008). However, identification of soybean lines carrying new aphid-resistance traits requires screening many lines; most of which are susceptible (e.g., Bansal et al. 2013; Hesler 2013; Bhusal et al. 2013, 2014; Hanson et al. 2016b). Furthermore, genetic mapping to determine the genetic basis of a resistance trait requires populations derived from lines known to have resistance and known susceptible parents while using genetic recombination in those lines to determine a region of a chromosome associated with the

resistance trait (e.g., Zhang et al. 2013). Both screening for identification of new resistance traits and follow-up phenotyping, which requires growing multiple generations for genetic mapping after an initial cross, can require considerable time and effort.

Management of soybean aphid with host-plant resistance is complicated by the fact that soybean aphid biotypes virulent to aphid-resistant plants continue to be discovered in North America (Kim et al. 2008b; Hill et al. 2012). Biotype 1 aphids cannot colonize plants with any known *Rag* genes, biotype 2 aphids can colonize plants with *Rag1* but not *Rag2*, biotype 3 aphids can colonize plants with *Rag2* (Hesler et al. 2013), and biotype 4 aphids can colonize plants with either *Rag1*, *Rag2*, or both genes (Alt and Ryan-Mahmutagic 2013). Additional sources of resistance will be needed to manage soybean aphid virulence to aphid-resistant plants (Michel et al. 2011; Hesler 2013; Hesler et al. 2013). Pyramiding multiple resistance genes further increases efficacy against soybean aphid (McCarville et al. 2014b; Chandrasena et al. 2015; Ajayi-Oyetunde et al. 2016).

Many of the lines previously used in screening studies were obtained from the United States Department of Agriculture's Soybean Germplasm Collection (Urbana, Illinois). The collection contains approximately 18,480 cultivated soybean lines (*Glycine max*) and 1,168 wild soybean (*G. soja*) lines (Song et al. 2015). Therefore, only about 18% of the collection has been screened and described in peer-reviewed literature to date. These 19,648 *G. max* and *G. soja* lines underwent a genotype analysis that identified 42,509 single nucleotide polymorphisms (SNPs) across the soybean genome for each line in the collection (Song et al. 2013, 2015).

With multiple screening studies for soybean aphid resistance and the genotype data provided by Song et al. (2015), it is possible to further explore the genetic basis of soybean aphid resistance without needing to develop mapping populations for each resistant line. Genome-wide association mapping studies (GWAS) rely on historical recombination events as opposed to recombination events from individual crosses of susceptible and resistant lines used to develop mapping populations (Myles et al. 2009). This type of analysis determines the correlation between genotype and phenotype at each SNP across multiple individuals. A high degree of correlation at a given SNP can indicate the SNP occurs within either a gene controlling the trait of interest or a locus closely linked to the gene; this approach has a high mapping resolution while examining a large number of alleles in the population (Myles et al. 2009). Multiple GWAS have identified agronomic traits for soybean including protein and oil content (Bandillo et al. 2015), flowering, maturity, and plant height (Zhang et al. 2015a), seed weight (Zhang et al. 2016), soybean sudden death syndrome resistance (Bao et al. 2015; Zhang et al. 2015b), Sclerotinia stem rot (Iquira et al. 2015), and soybean cyst nematode (Vuong et al. 2015). GWAS have also been used for aphid pests. Qin et al. (2017) examined cowpea resistance to cowpea aphid where 338 cowpea lines in the USDA Germplasm Collection with were used with 1,047 SNPs to detect two SNPs associated with cowpea aphid resistance.

We performed a GWAS analysis for soybean aphid resistance using phenotypic data across 2,366 soybean lines from previously published screening studies to facilitate future screening and breeding efforts. To guide future screening studies, we determined SNP markers that can be used to prioritize unscreened lines that may have underlying

genetics associated with aphid resistance. To guide future genetic mapping studies for confirmation of new *Rag* genes, we identified SNPs occurring in regions where *Rag* genes have not yet been documented.

3.2 Methods

3.2.1 Phenotype data

We reviewed the literature for studies screening for soybean aphid resistance using lines in the USDA Soybean Germplasm Collection. Screening methodologies were highly variable among studies, so we selected studies that had similar methodologies in order to pool data across studies. The methods for these studies generally consisted of potted plants of different soybean lines (early vegetative growth stages) being placed in close proximity to one another, so aphids could freely move between plants and aphid populations on the plants were allowed to grow for approximately 14 days. Such studies were chosen, because both antibiosis and antixenosis could influence aphid populations in these assays (Hanson et al. 2016b). Though lines have been identified exhibiting tolerance (Pierson et al. 2010; Bansal et al. 2013; Prochaska et al. 2013), such studies were not included in this analysis because too few lines have been screened for tolerance to provide a robust GWAS. In addition, tolerance studies measure differences in yield between infested and uninfested plants instead of measuring aphid densities (Prochaska et al. 2013).

Six studies were used to provide phenotype data (Table 3.2). In studies listed as using biotype 1 aphids, aphids came from a confirmed laboratory strain; those listed as biotype 2 or 3 were field collected colonies that overall exhibited biotype 2 or 3 reactions

in resistance assays, but may still contain other biotypes. Bhusal (2013 and 2014) studies were conducted in greenhouse conditions, and the remaining studies were performed in growth chambers. Excluding Hanson et al. (2016b), direct aphid counts were not available from these studies. Therefore, the reported aphid ratings for each line from the remaining studies were rounded to the nearest whole integer and converted to the midpoint of the respective scale's range (e.g., a rating encompassing 25 to 100 aphids would be converted 62.5 aphids) to account for interval-censoring and standardize the phenotype data across studies. Right-censored ratings (e.g., greater than 500 aphids) were approximated as 640 aphids for Bhusal et al. (2013, 2014), 500 aphids for Bansal et al. (2013) and 175 aphids for Hesler et al. (2013) and 275 aphids for Hesler et al. (2017). Country of origin (CO) and maturity group (MG) were obtained for each line from the USDA-GRIN database for further analysis (USDA-ARS 2016). In these six studies, 2,366 unique lines were present; 135 of these lines were included in multiple studies for 2,504 total phenotype ratings across all lines and experiments.

3.2.2 Genotype

Soybean SNP data was accessed from data made publicly available for download by Song et al. (2015) at https://soybase.org/snps/. For each soybean line, reads for each of 42,080 SNPs were coded as homozygous for either AT (1) or GC (-1) base pairs, heterozygous (0), or missing (NA). We excluded unanchored sequence scaffold SNPs from our analysis (Song et al. 2013). Some lines with known *Rag* genes included in the selected studies also had SNP data available. Bansal et al. (2013) used PI243540 (*Rag2*). Bhusal et al. (2013) included PI548663 (*Rag1*), PI243540 *(Rag2*), PI567543C (*Rag3*),

and PI 567541B (*rag4* and *rag1c*); these lines were also used by Bhusal et al. (2014) in addition to PI567598B (*rag1b*).

3.2.3 Genome-wide association mapping

Statistical analyses were conducted using R (v. 3.2.3). Associations between SNPs and aphid ratings were analyzed using the package *rrblup* for genome-wide association mapping using the mixed-model GWAS function (Endelman 2011). The package provides a p-value score (-*log*[*p*]) for each SNP across all soybean lines where scores above a multiple-comparisons-corrected false-detection threshold of $\alpha = 0.05$ indicate SNPs significantly correlated with aphid resistance. Aphid counts were log-transformed to adjust for non-normality and used as the response variable. Genotype was the primary explanatory variable (Martin et al. 2009; Schwantes-An et al. 2016). SNPs were not included in the analysis if their minor allele frequency (MAF) was < 0.05% (e.g., Bergfelder-Drüing et al. 2015) or if fewer than ten lines contained the minor allele to reduce the likelihood of false positive associations with aphid resistance while also avoiding exclusion of rare alleles (Tabangin et al. 2008). Kinship between lines was calculated by using the GWAS function to account for correlations that could occur due to individuals being closely related.

A fixed effect term was also included for each study to account for the different environments in which the studies were conducted, as well as the different rating scales used across the different studies. Initially, experiments were analyzed separately according to biotype with the biotype 1 analysis using study as a fixed-effect covariate to account for differences in methodology or rearing conditions between Bhusal et al.

(2013), Hesler (2013), Hanson et al. (2016b), and Hesler (2017). Data from all studies were also analyzed jointly with the GWAS function to determine SNPs significant across multiple biotypes by using the study fixed effect in the model to account for biotype differences.

Underlying population structure shared among lines, such as soybean species, maturity group, and country of origin was also considered by conducting a principle components analysis (PCA) using the FactoMineR package (Lê et al. 2008; Bandillo et al. 2015; Kumar et al. 2014). Principle components analysis was performed for lines included for each biotype to determine the number of principle components necessary to account for population structure in the GWAS analysis (Price et al. 2006; Husson et al. 2016). The number of principle components was determined by scree plot analysis for each separate biotype and all studies combined. To assess whether population structure explained variation in aphid counts on these lines before conducting the GWAS analysis, cluster analysis was performed using the daisy function in the cluster package (Maechler et al. 2016). ANOVA was performed with aphid rating as a response variable with study and cluster as covariates.

Also using R, a phylogenetic analysis was performed using neighbor-joining method in the ape package to determine the relatedness of each line in the included studies (Saito and Nei 1987; Paradis et al. 2004). Labels for each soybean line were coded in FigTree to indicate to which biotype the line was resistant to determine if resistance has occurred in multiple relatively unrelated lines or if resistance is isolated in a distinct group (Rambaut 2012).

3.2.4 Variation in SNPs associated with aphid resistance

For each individual biotype and the combined biotype analysis, the significant SNP with the highest *–log(p)* score within a 500 kb window was selected. Analysis of variance was used to examine the relative contribution of each significant SNP to aphid density variability in this analysis after adjusting for other covariates. Similar to the GWAS function, experimental environment of each study was included as a factor with log-transformed aphid counts as a response variable. Terms were also included for each SNP as a main effect to measure variance attributed to SNPs across all measured biotypes in addition to an interaction term to measure variance due to soybean genotype by aphid biotype interactions.

A haplotype block analysis was conducted in the software Haploview to determine the relative frequency of the allele associated with aphid resistance for selected significant SNPs (Barrett et al. 2005). The analysis was also used to determine if there was variation in resistance for haplotypes with the same nucleotide at the significant SNP. The four gamete method was used to determine block boundaries where recombination occurs between adjacent SNPs (Wang et al. 2002). A minimum haplotype frequency of 0.5% was used unless the significant SNP was monomorphic for all haplotypes above 0.5%

3.3 Results

Within each study, there were few lines with relatively lower aphid counts compared to those with higher aphid counts (Fig. 3.1). From principle components analysis, the first six principle components accounted for 25.2% of genetic structure, with

the first two components accounting for 9.4% and 6.3% of variance, respectively (Fig. 3.2). Among species, country of origin, and maturity group, the variance explained by the first dimension was most correlated with species as an explanatory variable (r^2 = 0.74), and country of origin (r^2 = 0.49) accounted for the variance in dimension two more than maturity group (r^2 = 0.35). In the cluster analysis, four clusters were identified and assigned to each soybean line. Cluster was significant [F (3, 2458) = 6.858; $SS_{ckyster}$=1.82; SS_r = 446.61; p = 0.018], but explained relatively little additional variance compared to study [F (5, 2458) = 270.25; SS_{study} = 245.52; SS_r = 446.61; p < 0.001], which indicated population structure had a minor but significant effect on phenotypes for lines in this study. (Fig. 3.3)

Individual resistance to biotypes 1, 2, and 3 appeared to occur in relatively distantly related individuals for lines within this study (Fig. 3.3). It was also not uncommon to find resistance documented for closely related individuals, especially for resistance to multiple biotypes. PI6125759C and PI612759B are resistant to biotype 1 and 2, respectively, and were grouped on adjacent nodes (Fig. 3.3). PI639537 resistant to biotype 1 and PI437075 resistant to biotype 3, both originating from Russia, were also similarly grouped (Fig. 3.3). PI340941, PI567250A (CO: China; M.G.: I), and PI603712 (CO: China; M.G.: 0) were another group of adjacent nodes, which had biotype 2, biotype 3, and biotype 2 and 3 resistance, respectively (Fig. 3.3). Other closely related lines included PI567598B with biotype 3 resistance, and PI567597C and PI567543C with biotype 2 and 3 resistance, each of which were maturity group III lines originating from China (Fig. 3.3). While not as closely related as previously mentioned groups, PI157492 (CO: Japan; MG: IV) and 605765B (CO: Vietnam MG: unknown) with biotype 1

resistance, PI567541B (CO: China; MG: III) with biotype 1 and 3 resistance, and PI603587A (CO: China; MG: I) with biotype 2 and 3 resistance occurred within a set of higher level branches (Fig. 3.3).

3.3.1 Genome-wide association mapping

Manhattan plots for biotype 1 aphids on *G. max* and *G. soja* lines show SNPs were highly significantly correlated with soybean aphid population density at chromosomes 2, 7, and 13 (Fig 3.4). Significant SNPs were also present on chromosomes 2, 5, 9–11, and 16–20 where no aphid resistance genes have been documented to date (Fig. 3.4). One significant SNP on chromosome 7 fell within the range of *rag1c* (Tables 3.1; Fig. 3.1), but other significant SNPs occurred outside the range of known *Rag1* and *rag1* genes. Two peaks occurred on chromosome 13 where multiple resistance genes are present (Fig. 3.4). The most significant SNP on chromosome 13 fell within the range of *Rag2* or *Rag5*, but the second peak occurred approximately 45 Mbp from this region (Fig. 3.4).

Significant SNPs were also found for biotype 2 aphids that overcome *Rag1* from Bhusal et al. (2013) on chromosomes 1, 4, 6, 8, 10, 12, 13, and 14 (Fig. 3.5a). Significant SNPs on chromosomes 7 and 13 fell within the range of known *Rag* gene (Fig 3.5a). The significant SNP on chromosome 7 at 5,062,637 bp was close to the range of the *Rag1* and *rag1b* while also within the relatively wide range for *rag1c*; a second SNP on chromosome 7 was at least 28.6 Mbp from these genes (Table 3.1).

For aphids exhibiting biotype 3 characteristics or being able to overcome *Rag2* from Bhusal et al. (2014), significant SNPs on chromosomes without known soybean

aphid resistance genes were found on chromosomes 5, 8, 10, and 19 (Fig 3.5a). Significant SNPs were also found on chromosome 13 outside the range of known *Rag* genes (Fig. 3.5a). The SNP on chromosome 8 at 41,031,762 bp was about 424 kbp from *Rag6.*

In the combined analyses of all biotypes used in the studies we examined, significant SNPs were found associated with aphid densities across biotypes on chromosomes 1, 5, 6, 18, and 19 where *Rag* genes have not been documented (Fig. 3.6). A significant SNP was also found on chromosome 13 within the range of *Rag2* and *Rag5,* and another was approximately 2.5 Mbp outside this range (Fig. 3.6). Additionally, some SNPs were significant in multiple analyses. Two SNPs were detected in both the biotype 1 analysis and all biotypes combined on chromosome 13 and one SNP on chromosome 18 (Table 3.3). Another SNP was significant on chromosome 5 for biotype 1, 3, and combined analyses (Table 3.3).

3.3.2 Variation in SNPS associated with aphid resistance

ANOVA indicated 60.7% of variation in all experiments included in this analysis was explained by genetic factors (i.e., differences in alleles at a given SNP) and other covariates. Study or environment explained 32.9% of the variance in aphid density, but this effect was also confounded with the main effect of biotype since each study we analyzed did not include more than one biotype. The main effect of genotype accounted for 12.9% of the phenotypic variance, and genotype by biotype interactions explained an additional 15.0% of the variance.

Of the variance accounted for by the main effect of genotype, SNPs within ranges of known *Rag* genes accounted for 13.8% of this variation, which was primarily due to SNPs within the *Rag2* and *Rag5* ranges (Table 3.3). SNPs on chromosomes 6, 10, and 20 each explain greater than an additional 5% variation and are not in close proximity to known *Rag* genes (Table 3.3). Of the variance accounted for by genotype by biotype interactions or biotype specific variation, known SNPs within the regions of known *Rag* genes accounted for 14.1% of variance. SNPs on chromosomes 1, 5, 6, and 16 without *Rag* genes accounted for 26.1% of biotype specific variation (Table 3.3). The effect size of alleles at each SNP also varied depending upon biotypes (Fig. 3.7)

Resistant haplotypes ranged between 0.3 and 17.4% frequency. Resistant haplotypes typically in less common haplotypes (i.e., below 0.05%, haplotype frequency) but two haplotypes on chromosome 5 and 13 (e.g., H2 near ss715590836 and H2 near ss715615008, respectively) occurred at 11.1 and 17.4%, respectively (Table 3.4). No block was found for ss715615024 on chromosome 13. In some cases, such as the block on chromosome 13 containing ss715614932, multiple haplotypes contained the allele associated with resistance in the significant SNP, but H6 did not have fewer aphids than H5 (Table 3.4). This difference also occurred on chromosome 20 for the block containing ss715637718 (Table 3.4). In regions where GWAS determined a SNP was significant in the combined biotype analysis on chromosome 1, 5, 6, 13, and 18 (Table 3.3), differences in aphid densities were seen across biotypes by haplotype (Table 3.4). Haplotypes containing these SNPs typically showed decreased biotype 2 and 3 densities and a marginal decrease in biotype 1 aphid densities (Table 3.4).

3.4 Discussion

We analyzed data from six published studies and examined the phylogeny and population structure of lines screened in those studies. PCA indicated species and country origin appeared to be primary sources of population structure (Fig. 3.2), which was similar to findings by Bandillo et al. (2015). Phylogenetic analysis showed that resistance to individual biotypes was often found in relatively unrelated individuals and not clustered only in closely related groups of individuals (Fig. 3.3). However, resistance to multiple biotypes often did occur in closely related groups of individuals (Fig. 3.3), which may indicate ancestors of those groups underwent strong selection pressure to develop multi-biotype resistance. These multi-biotype resistant groups may be a priority for additional linkage mapping experiments (e.g., Zhang et al. 2013; Bhusal et al. 2017) or assessing how such resistance develops in conjunction with soybean aphid biotype evolutionary development (Michel et al. 2011).

In the GWAS analysis, 45 SNPs significantly correlated with soybean aphid resistance on 18 of the 20 soybean chromosomes for the three individual biotypes and across biotypes. Significant SNPs were found on chromosomes 7, 8, 13, and 16 with known *Rag* genes (Table 3.1; Table 3.3). SNPs were also significant on chromosomes 1, 2, 4, 5, 6, 9, 10, 11, 12, 14, 17, 18, and 19, and 20 where *Rag* genes have not yet been mapped (Table 3.3). *Rag1*, *rag1b*, and *rag1c* have been mapped to similar regions on chromosome 7 (Zhang et al. 2009; Kim et al. 2010a; Bales et al. 2013). Significant SNPs from this analysis also fell either within or close to these regions. However, ss715598285 was also significant against biotype 2 populations (Table 3.3), which may indicate the presence of a non-*Rag1* gene associated with this region on chromosome 7. For biotype 3 aphids, significant SNPs were not found within the range of *Rag2* on chromosome 13.

Ss715614932 on chromosome 13 accounted for a large amount of variation in aphid density regardless of biotype. This region is associated with multiple known *Rag* genes, especially *Rag5* that has no known virulent biotype (Table 3.2). Other SNPs, such as ss715579738 on chromosome 5, did not account for a large amount of variation overall, but instead accounted for a large amount of variation for specific biotypes. Ss715578827 on chromosome 1 also had a large amount of variation in resistance to specific biotypes, but this SNP was only significant when all biotypes were analyzed jointly (Table 3.3). This would seem to indicate that while this SNP is associated with differences in aphid densities across all biotypes rather than one biotype, the magnitude of those differences may vary depending upon biotype as seen when the haplotypes containing theses SNPs are analyzed further (Table 3.4).

Some significant SNPs from the GWAS analysis, such as ss715583602 on chromosome, 2 did not account for large amounts of genetic variation either for main or interaction effects in the follow-up ANOVA (Table 3.3). A combination of two factors could cause instances such as this. Alleles at each SNP could range from common to rare where a rarer resistance allele could result in that SNP accounting for less variation in the overall population even if it has a strong effect. Conversely, an allele may have a weak effect while having a high allele frequency. In this case, ss715583602 has a rarer MAF of 0.5%. Marker effect size often has in inverse relationship with allele frequency (Lettre 2011; Park et al. 2011).

Other SNPs, such as ss715578827, were significant in the combined biotype analysis, but had a small effect size (Fig. 3.7) with a 3.8% MAF. Because GWAS is prone to low power (Spencer et al. 2009), this SNP was likely not detected in individual

biotype analyses with smaller sample sizes. The haplotype block analysis also confirmed that alleles associated with resistance are uncommon (Table 3.4). This may indicate weak but broad, non- specific resistance (i.e., horizontal resistance) as it was a significant SNP across biotypes (Van Der Plank 1966). Horizontal resistance would be more difficult to detect in soybean aphid screening assays due to a lower effect size, and only vertical resistance (i.e., biotype specific resistance) has been documented to date (Hesler 2013). Horizontal resistance has been found in other aphid species (e.g., Nielson and Kuehl 1982). The benefit of our combined biotype analysis (Fig. 3.6) is the increased sample size and power to detect these potential effects that may not be detected in the single biotype analyses. In this analysis, potential horizontal resistance would be associated with significant SNPs found in the combined analysis that account for a relatively high amount main effect genetic variability with low variance for biotype interactions. If horizontal resistance can be found for soybean aphid, it would be a valuable tool in maintaining effective host-plant resistance to combat biotypes. (Smith and Chuang 2014)

Chang and Hartman (2017) also performed a GWAS on soybean aphid resistance in the USDA germplasm collection using 2,395 lines, but only found one SNP, ss71559614 on chromosome 7, that was significantly associated with resistance. This is a marked difference compared to the number of SNPs we detected. The datasets we analyzed, excluding Bhusal (2013), where not examined by Chang and Hartman (2017), so it is difficult to make direct comparisons between studies. However, Chang and Hartman (2017) used categorical phenotypic data on the USDA-GRIN database instead of using aphid densities or rating scales reported in the literature. This would comparatively reduce the resolution of their phenotypic data and their power to detect

underlying resistance. Chang and Hartman (2017) also used studies with different biotypes, but did not indicate if biotype or experiment effects were accounted for in the GWAS model. Not accounting for experiment or biotype as part of a meta-analysis could obscure the significance of individual SNPs (Cornelis et al. 2010).

There are few GWAS studies of soybean related insects pests or other pests such as nematodes, and those that have examined soybean pests have generally found few SNPs associated with resistance (Wang et al. 2015; Vuong et al. 2015; Liu et al. 2016; Chang and Hartman 2017). Due to the number of significant SNPs found in this study for each biotype, there appears to be significant genetic variation associated with soybean aphid resistance. This could either indicate that multiple undescribed soybean aphid resistance genes are present in the lines we examined, but multiple SNPs could also be correlated with a single resistance trait. From the SNP markers provided in this analysis, researchers may be able to pre-screen for currently genotyped lines in the USDA Germplasm Collection that are likely to have resistance traits for additional aphid resistance assays. This will allow researchers to focus their efforts on lines that have a high potential to have traits associated with aphid resistance rather than screening many random susceptible lines. However, having a line that is not correlated with resistant sources examined in this study does not necessarily imply the line is susceptible. These markers could also be used in future studies to determine if they would be of use marker-assisted selection (Collard et al. 2005).

While many lines in the USDA Germplasm Collection have not yet been screened for resistance, many lines that do have confirmed resistance have not undergone mapping experiments. Resistant soybean lines that also have alleles in regions associated with

resistance identified in this study without known *Rag* genes could be prioritized for future linkage mapping experiments (e.g., Hill et al. 2006; Zhang et al. 2013). Our identification of SNPs associated with soybean aphid resistance should provide a new resource to guide researchers in future soybean aphid screening and mapping experiments and will hopefully expedite the discovery and integration of additional soybean aphid resistance genes into available soybean varieties.

3.5 Tables

Table 3.1. Known *Rag* genes and the approximate base pair (bp) position range of the closest reported markers flanking the gene.

Rag gene	Chromosome	Position range (bp)		Reference
Rag1	7	5,529,532	5,770,718	Kim et al. 2010a
rag1b (provisional)	7	5,523,128	5,909,485	Bales et al. 2013
rag1c	7	2,434,259	8,234,168	Zhang et al. 2009
Rag2	13	29,609,521	31,802,676	Kim et al. 2010b
Rag3	16	4,964,852	7,212,164	Zhang et al. 2010
rag3 (provisional)	16	6,314,120	6,570,336	Bales et al. 2013
rag3b	16	4,964,852	7,957,026	Zhang et al. 2013
rag4	13	1,225,665	16,340,514	Zhang et al. 2009
Rag5 (provisional)	13	30,236,183	30,749,047	Jun et al.2012
Rag6 (provisional)	8	39,041,088	40,607,489	Xiao et al. 2013

Positions of reported flanking markers were obtained from the *Glycine max* genome assembly (Glyma 2.0): https://soybase.org/gb2/gbrowse/gmax2.0. In cases where a marker location was not listed in Glyma 2.0, the position of a nearby marker was reported as an approximation.

Table 3.2. Studies used for aphid-resistance phenotype data assessed at 14 days after infestation and that include lines with SNP data.

Species	Study	Lines	Maturity groups	Biotype	State	Scale
G. max	Bansal et al. 2013 (Supp. Table 1)	873	II – IV	1	OH	1 to 5 rating scale: 1 < 25, 2 = 25-100, 3 = 101-200, 4 = 201-400, and 5 > 400 aphids per plant
	Bhusal et al. 2013 (Table 3)	334	I	2*	SD	scale similar to Bansal et al. (2013) except a 4 = 201-500 and 5 >500.
	Bhusal et al. 2014 (Suppl. 1)	341	00 – 0	3†	SD	same scale as Bhusal et al. (2013)
	Hanson et al. 2016b (Fig. 1)	74	000 – I	1	MN	aphid counts per plant
	Hesler et al. 2017	745	0 – I	1	SD	1 to 6 rating scale: 1 < 51, 2 = 51-100, 3 = 101-150, 4 =151-200, 5 = 201-250, and 6 >250 aphids per plant
G. soja	Hesler 2013 (Table 1)	137	0 – III	1	SD	1 to 4 rating scale: 1 < 51, 2 = 51-100, 3 = 101-150, and 4>150 aphids per plant

*Field collected population virulent on a *Rag1* line, but avirulent on *Rag2*

† Field collected population virulent on a *Rag2* line, but avirulent on *Rag1*

Table 3.3. Genetic variation accounted for by all significant SNPs and interactions with soybean aphid biotypes. SNPs within reported ranges of known *Rag* genes are in bold.

SNP ID	Chr.	Location (bp)	Genotype[1]	Gen. x Biotype[1]	MAF	Biotype[2]
ss715578827	1	2,637,003	2.22%	6.63%	0.038	All
ss715580619		55,775,590	0.33%	1.10%	0.146	B2
ss715583602	2	5,475,047	0.11%	0.00%	0.005	B1
ss715589122	4	6,142,596	0.67%	0.23%	0.075	B2
ss715590206	5	24,133,841	0.22%	7.98%	0.008	B1
ss715590836		33,212,449	2.19%	0.48%	0.127	B1,B3,All
ss715590997		34,337,698	0.08%	0.67%	0.124	B3
ss715594602	6	46,884,182	7.34%	6.50%	0.033	All
ss715594619		46,950,450	0.98%	3.93%	0.099	B2
ss715596585	7	1,671,208	0.12%	0.00%	0.028	B1
ss715596894		**2,530,979**	**2.82%**	**0.36%**	**0.041**	**B1**
ss715598285		**5,062,637**	**0.53%**	**0.37%**	**0.165**	**B2**
ss715597329		35,436,934	1.11%	0.78%	0.022	B1
ss715599482	8	13,783,090	0.18%	1.22%	0.093	B2
ss715599561		14,338,011	4.18%	1.60%	0.186	B2
ss715600535		20,464,889	4.96%	5.73%	0.034	B1
ss715600829		22,052,131	1.98%	1.45%	0.059	B1
ss715601800		41,031,762	0.70%	4.07%	0.03	B3
ss715603059	9	1,431,512	3.00%	0.00%	0.022	B1
ss715606645	10	38,676,101	6.12%	1.98%	0.005	B1
ss715607270		43,371,238	1.78%	2.77%	0.055	B1
ss715607701		47,716,772	0.01%	1.13%	0.269	B2
ss715608208		51,462,329	0.45%	0.36%	0.035	B3
ss715609271	11	25,347,421	1.86%	0.00%	0.004	B1
ss715612718	12	36,995,143	0.03%	0.49%	0.15	B2
ss715614449	13	27,392,456	0.23%	2.99%	0.092	B3
ss715614803		29,459,954	2.04%	2.04%	0.085	B1
ss715614932		**30,186,161**	**15.89%**	**7.65%**	**0.031**	**B1,All**
ss715615008		**30,654,291**	**5.52%**	**1.46%**	**0.175**	**B2**
ss715615024		**30,724,301**	**4.88%**	**3.52%**	**0.19**	**B1,All**
ss715615352		32,859,112	1.39%	1.80%	0.041	B3
ss715615402		33,280,297	1.29%	1.27%	0.131	All
ss715616460		43,544,806	1.16%	0.86%	0.095	B3
ss715616609		45,558,151	3.38%	0.00%	0.007	B1
ss715617401	14	10,274,971	0.34%	0.39%	0.15	B2
ss715618940		43,805,410	0.90%	4.71%	0.177	B2
ss715625258	**16**	**6,093,779**	**0.22%**	**0.77%**	**0.041**	**B1**
ss715624134		29,528,105	2.69%	5.01%	0.017	B1
ss715628067	17	5,888,944	2.10%	1.84%	0.472	B1
ss715631460	18	49,223,187	0.97%	4.85%	0.042	B1,All
ss715634601	19	228,660	0.00%	0.00%	0.045	B3
ss715635565		46,220,139	0.36%	2.83%	0.175	B3
ss715635663		47,348,833	3.88%	3.56%	0.124	B1
ss715635693		47,552,973	3.28%	4.62%	0.015	All
ss715637718	20	36,626,029	5.50%	0.00%	0.038	B1

[1] Percentages are calculated by dividing the sum of squares (type II) for each main effect and interaction effect per SNP by the sum of squares across all SNPs and interaction effects.

[2] Biotype indicates the analysis in which the SNP was significant.

Table 3.4. Haplotype frequencies of selected significant SNPs for soybean aphid resistance to biotypes examined in this study. Bolded nucleotides the position of the SNP identified by GWAS.

						Biotype mean aphids		
Chr.	SNP	Block position (Mb)	Hapl. #	Haplotype	AF	1	2	3
1	ss715578827	2.637 – 2.715	H1	**T**TCTC	0.649	302	253	257
			H2	**T**TTCC	0.265	294	240	245
			H3	**T**TCCC	0.036	312	275	320
			H4	**C**TTCC	0.028	328	12	28
			H5	**C**CTCC	0.008	251	-	-
			H6	**T**TTTC	0.005	334	150	-
5	ss715590206	23.954 – 24.314	H1	AAGC**A**CAGTG	0.676	300	247	235
			H2	AAGC**A**CGGTG	0.188	305	253	294
			H3	AAGC**A**CAGTA	0.072	311	327	287
			H4	GCGC**A**CGGTG	0.05	315	-	-
			H5	GCGC**G**CGGTG	0.006	175	63	13
	ss715590836	33.21 – 33.213	H1	TA**A**G	0.626	315	238	270
			H2	CA**A**A	0.224	302	293	279
			H3	TC**G**G	0.111	265	198	130
			H4	TA**A**A	0.021	304	311	270
6	ss715594602	46.85 – 46.884	H1	TTCCAATCACG**T**	0.452	298	253	286
			H2	CCTTGGCCGTG**T**	0.373	304	260	256
			H3	TTCCAATAGCT**T**	0.08	303	301	212
			H4	TTCCAATCGCG**T**	0.035	300	182	228
			H5	TTCCAATCACG**C**	0.024	270	55	94
			H6	CCTTGGTCGTG**T**	0.018	296	351	230
			H7	CCTTGGCCGTG**C**	0.006	265	151	-
7	ss715596894	2.492 – 2.533	H1	CGC**G**A	0.722	319	254	257
			H2	TGC**G**G	0.099	304	216	275
			H3	TAT**G**G	0.065	310	207	210
			H4	TAC**G**G	0.046	269	-	-
			H5	TAT**A**G	0.031	290	331	250
			H6	TGC**G**A	0.018	291	62	87
			H7	TAT**G**A	0.007	345	66	248
8	ss715600535	20.441 – 20.494	H1	C**C**CT	0.592	312	239	243
			H2	C**C**CC	0.299	326	249	261
			H3	T**C**TT	0.044	302	322	192
			H4	C**C**TT	0.03	305	319	330
			H5	T**A**TT	0.029	231	350	350
10	ss715606645	38.64 – 38.676	H1	TACA**C**	0.506	306	260	260
			H2	CCCA**C**	0.256	290	232	238
			H3	CCCG**C**	0.144	307	224	257
			H4	CACA**C**	0.087	306	229	301
			H5	CCAA**T**	0.003	107	-	63

Chr.	SNP	Block position (Mb)	Hapl. #	Haplotype	AF	Biotype mean aphids		
						1	2	3
13	ss715614932	30.167 – 30.233	H1	CACG**C**GC	0.496	305	245	259
			H2	TGTA**C**GC	0.233	312	253	226
			H3	TGTA**C**AT	0.146	318	257	272
			H4	CACA**C**GC	0.084	326	284	273
			H5	CACA**T**GC	0.022	164	33	43
			H6	TGTA**T**GC	0.007	318	-	-
			H7	TACG**C**GC	0.007	328	-	-
	ss715615008	30.652 – 30.654	H1	A**T**	0.792	305	262	254
			H2	G**C**	0.174	286	102	179
			H3	G**T**	0.033	322	296	350
16	ss715625258	6.094 – 6.105	H1	**T**TC	0.4	324	256	256
			H2	**T**TT	0.39	311	251	253
			H3	**T**CT	0.169	289	255	280
			H4	**C**CT	0.04	217	233	177
	ss715625258	29.52 – 29.538	H1	CT**C**GA	0.412	305	182	258
			H2	TC**C**AA	0.4	322	253	254
			H3	TT**C**GG	0.158	309	275	250
			H4	TT**T**GG	0.012	207	-	-
			H5	TT**C**GA	0.011	206	351	211
18	ss715631460	49.223 – 49.256	H1	**A**TTTGC	0.631	303	258	266
			H2	**A**CCGAC	0.211	301	223	248
			H3	**A**CCTGC	0.094	309	304	232
			H4	**C**CCTGA	0.04	267	87	123
			H5	**A**TCGAC	0.006	314	-	-
19	ss715635693	47.553 – 47.637	H1	**A**ATCCCAG	0.424	316	246	279
			H2	**A**ATTTAAG	0.254	313	275	224
			H3	**A**CCCCCCA	0.235	300	176	200
			H4	**A**ATTTCAG	0.03	312	230	284
			H5	**A**ATTCCAG	0.022	226	351	350
			H6	**G**CCCCCCA	0.015	318	304	350
20	ss715637718	36.622 – 36.652	H1	G**T**AAAA	0.402	303	266	279
			H2	G**T**CAGC	0.291	289	251	226
			H3	G**T**CAGA	0.188	324	108	236
			H4	A**T**CAGA	0.053	306	201	213
			H5	G**C**CAGC	0.034	293	350	-
			H6	G**T**CGGA	0.017	276	66	148
			H7	G**C**CAGA	0.005	227	-	-

3.6 Figures

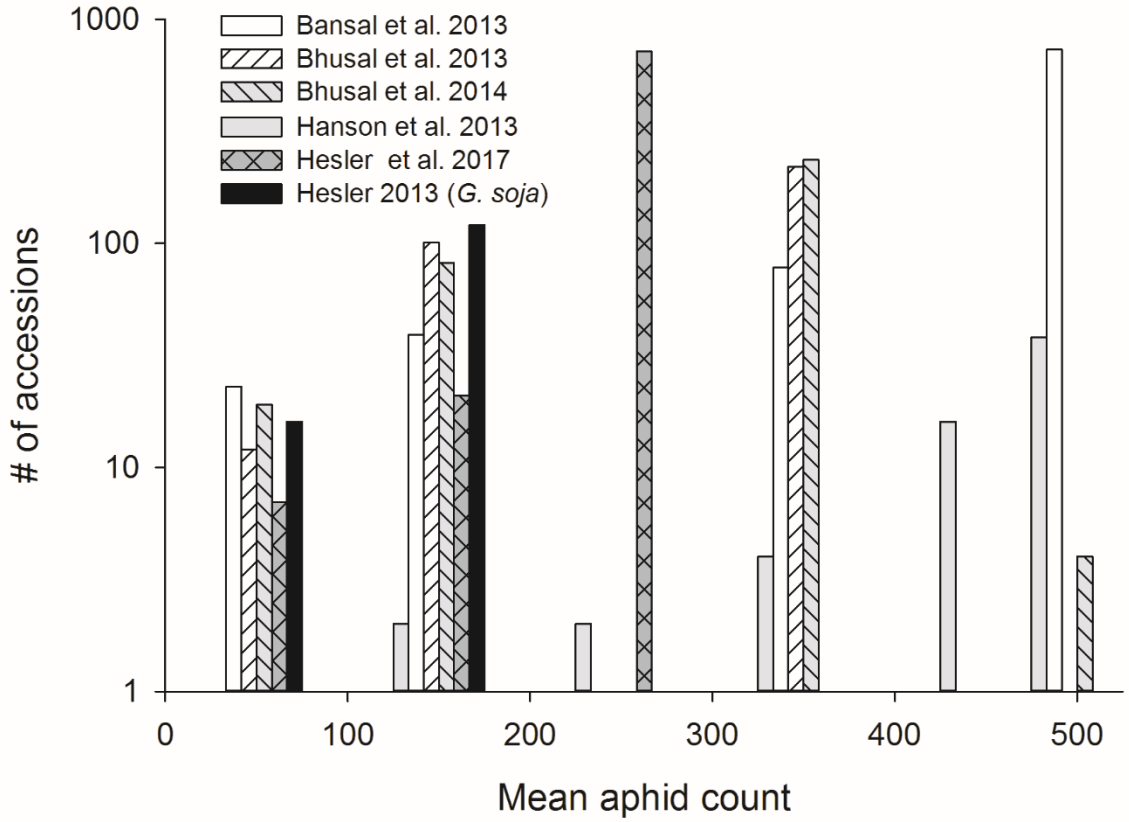

Figure 3.1. Number of accessions for each approximate range (e.g., 0 – 100) of mean aphid counts determined from direct count data or rating scales depending on study methodology.

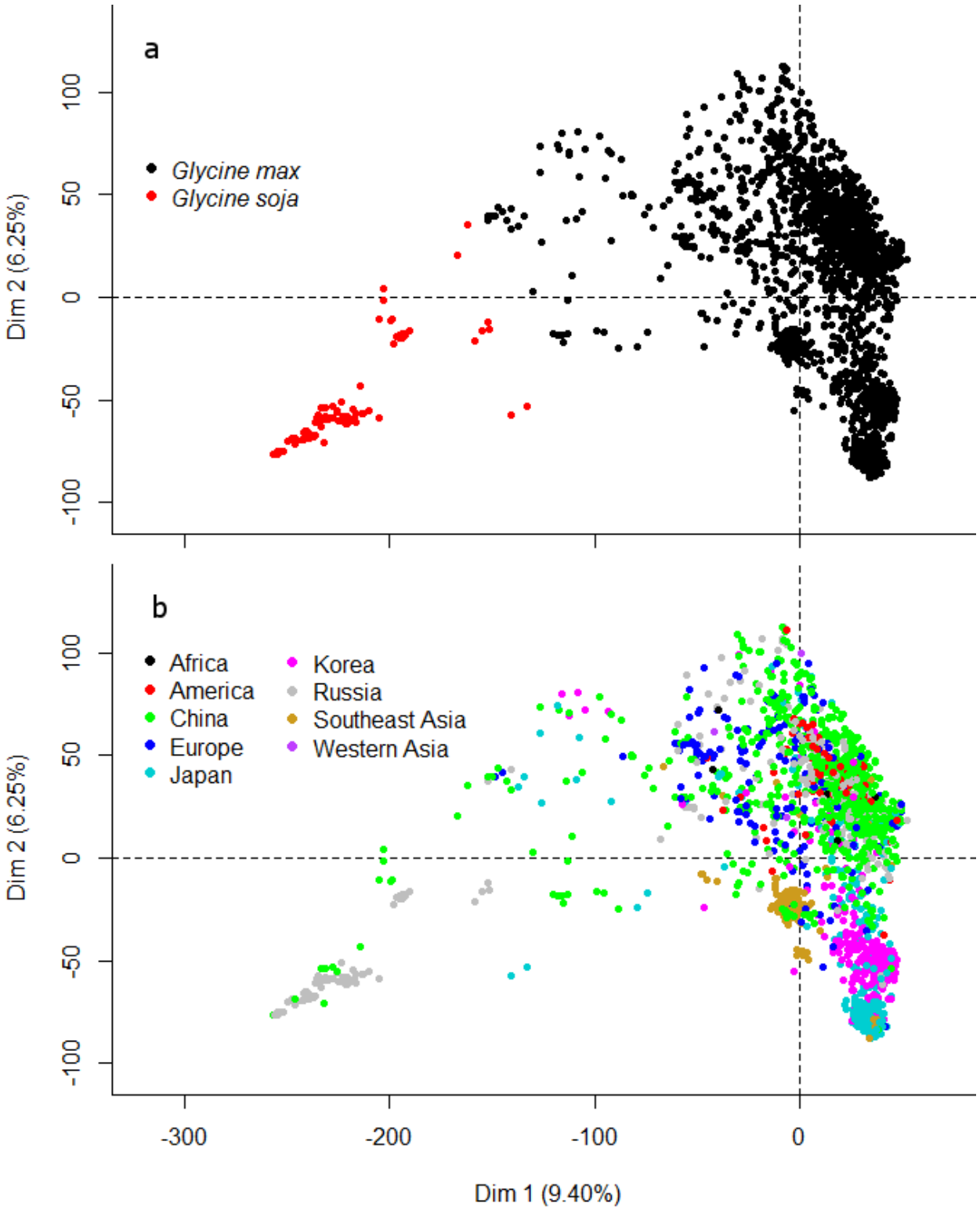

Figure 3.2. Principle components analysis categorized by **a)** soybean species and **b)** country or region of origin.

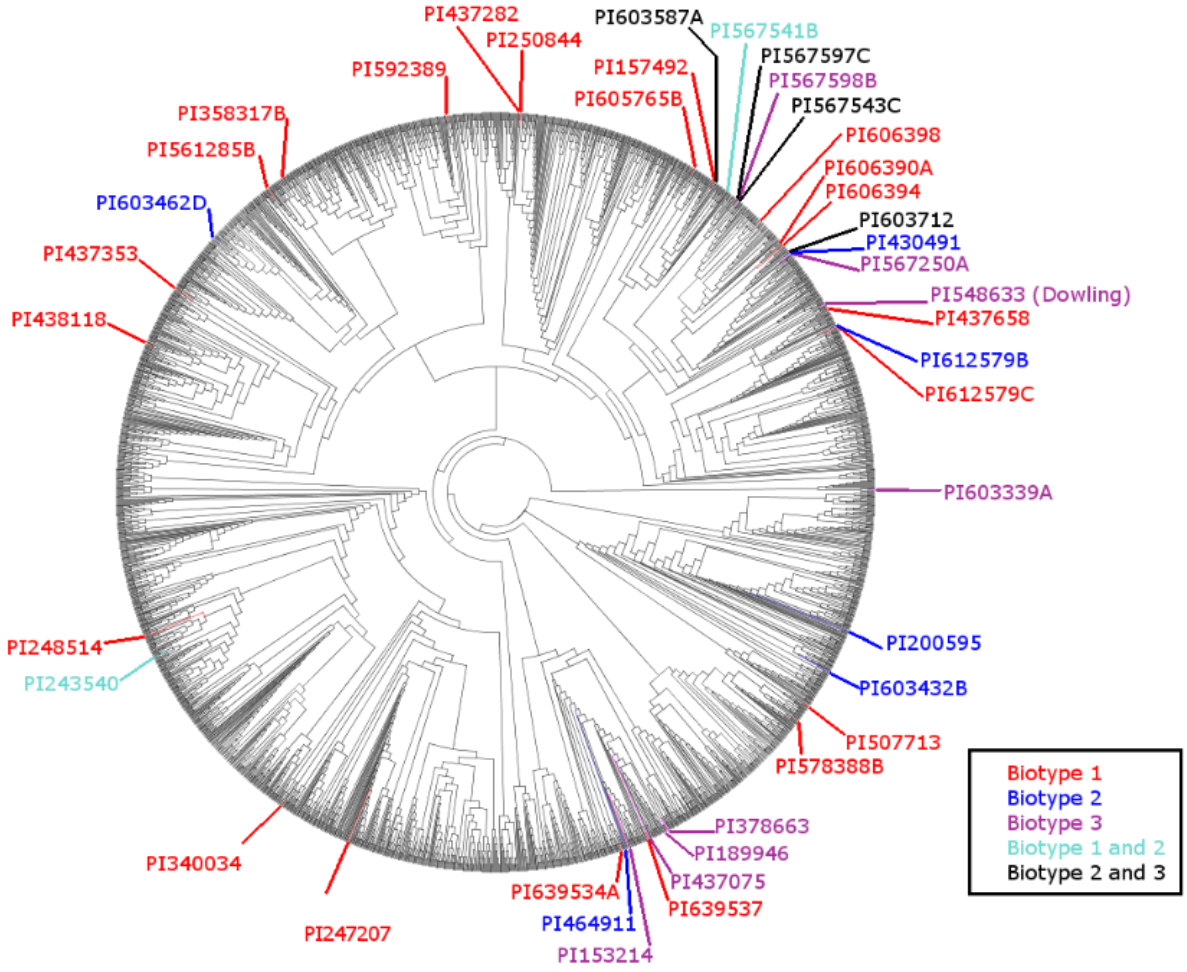

Figure 3.3. Phylogenetic tree constructed by neighbor-joining to show the relatedness of the 2,366 soybean lines from studies included in the GWAS analysis. Colored line names near leaf tips indicate lines with documented resistance to individual soybean aphid biotypes and resistance to multiple biotypes when reported by multiple studies.

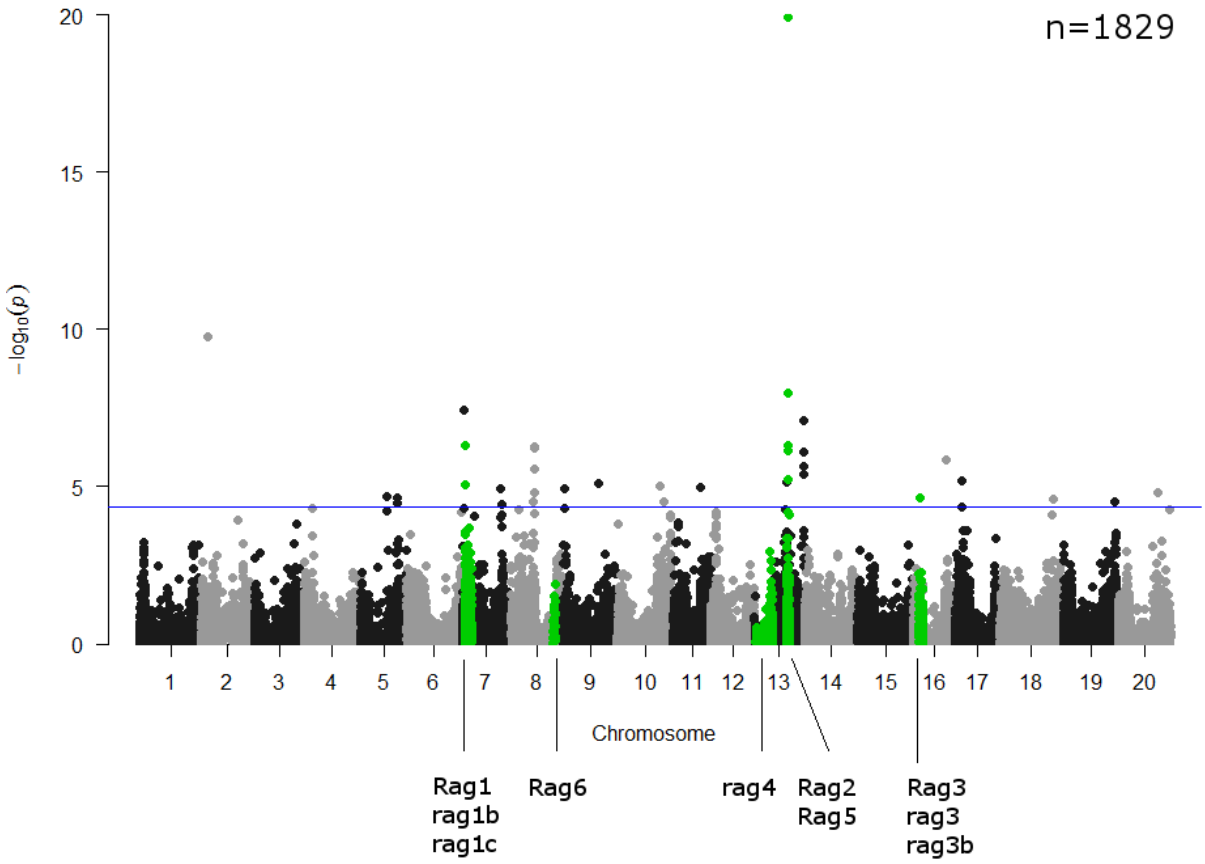

Figure 3.4. Correlation of SNPs and counts per plant for biotype 1 soybean aphids in combined *Glycine max* and *G. soja* experiments**.** SNPs above the solid line indicate a significant correlation with aphid density rating.

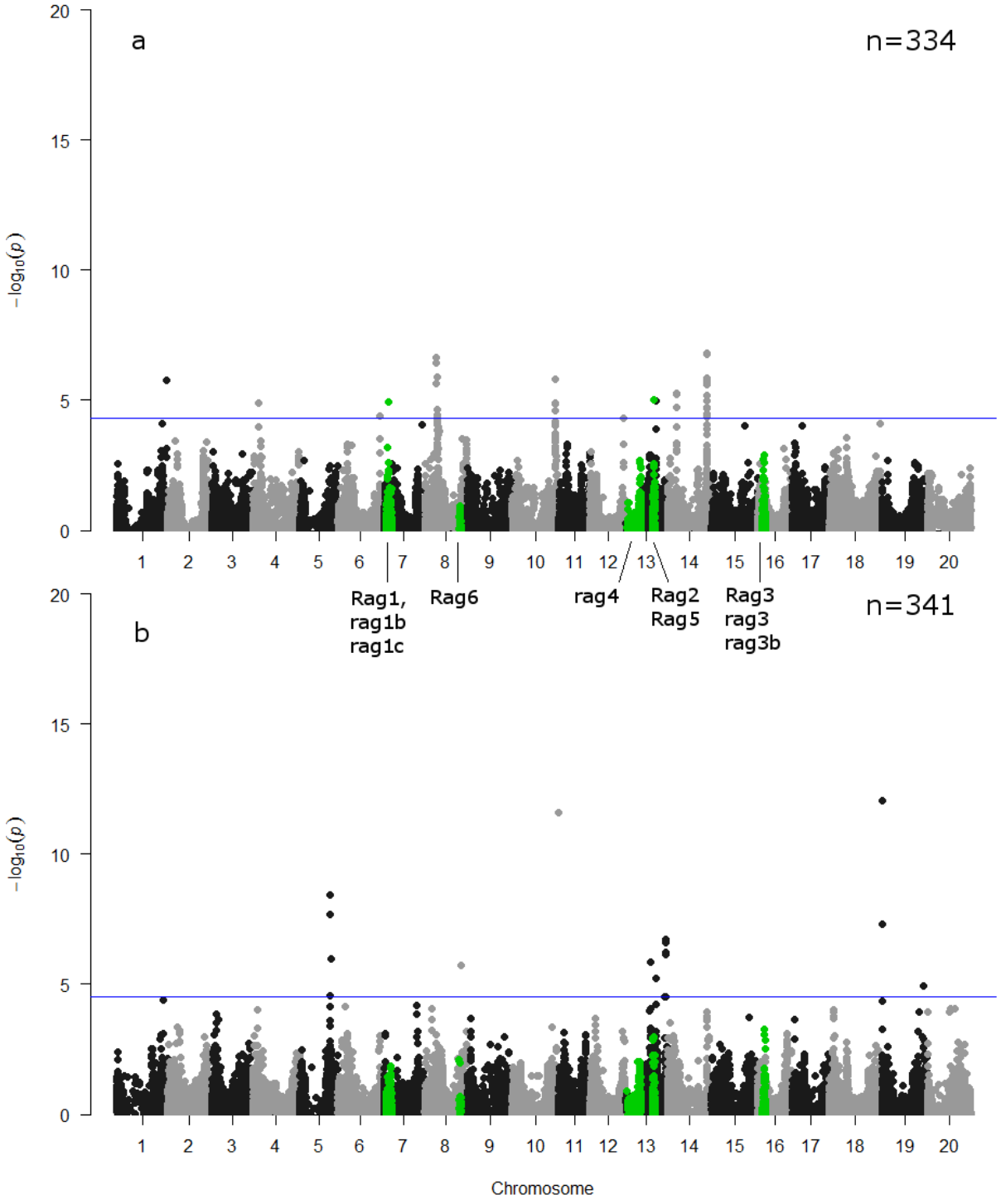

Figure. 3.5. Correlation of SNPs and counts per plant for **a)** biotype 2 soybean aphids **b)** biotype 3 soybean aphids. SNPs above the solid line indicate a significant correlation with aphid density rating.

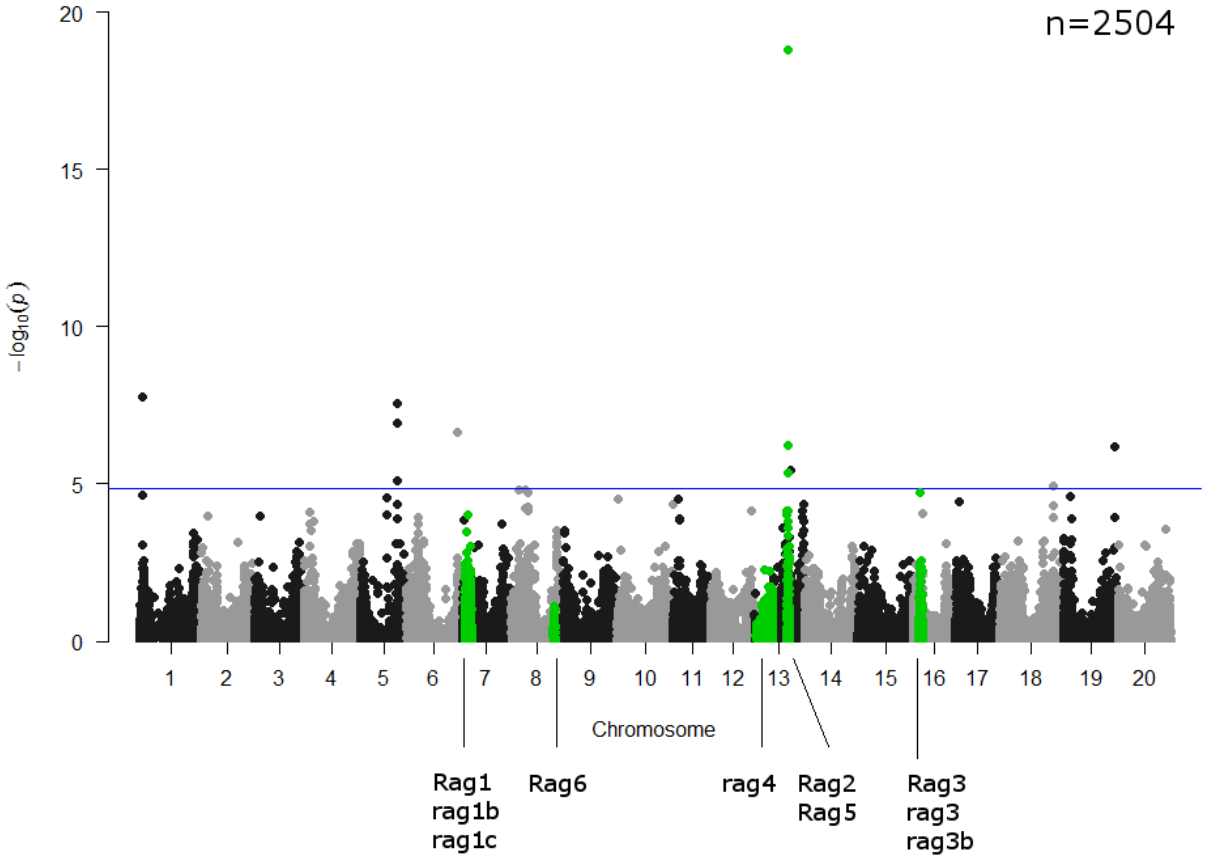

Figure. 3.6. Correlation of SNPs with density ratings across soybean aphid biotypes 1, 2 and 3**.** SNPs above the solid line indicate a significant correlation with aphid density rating.

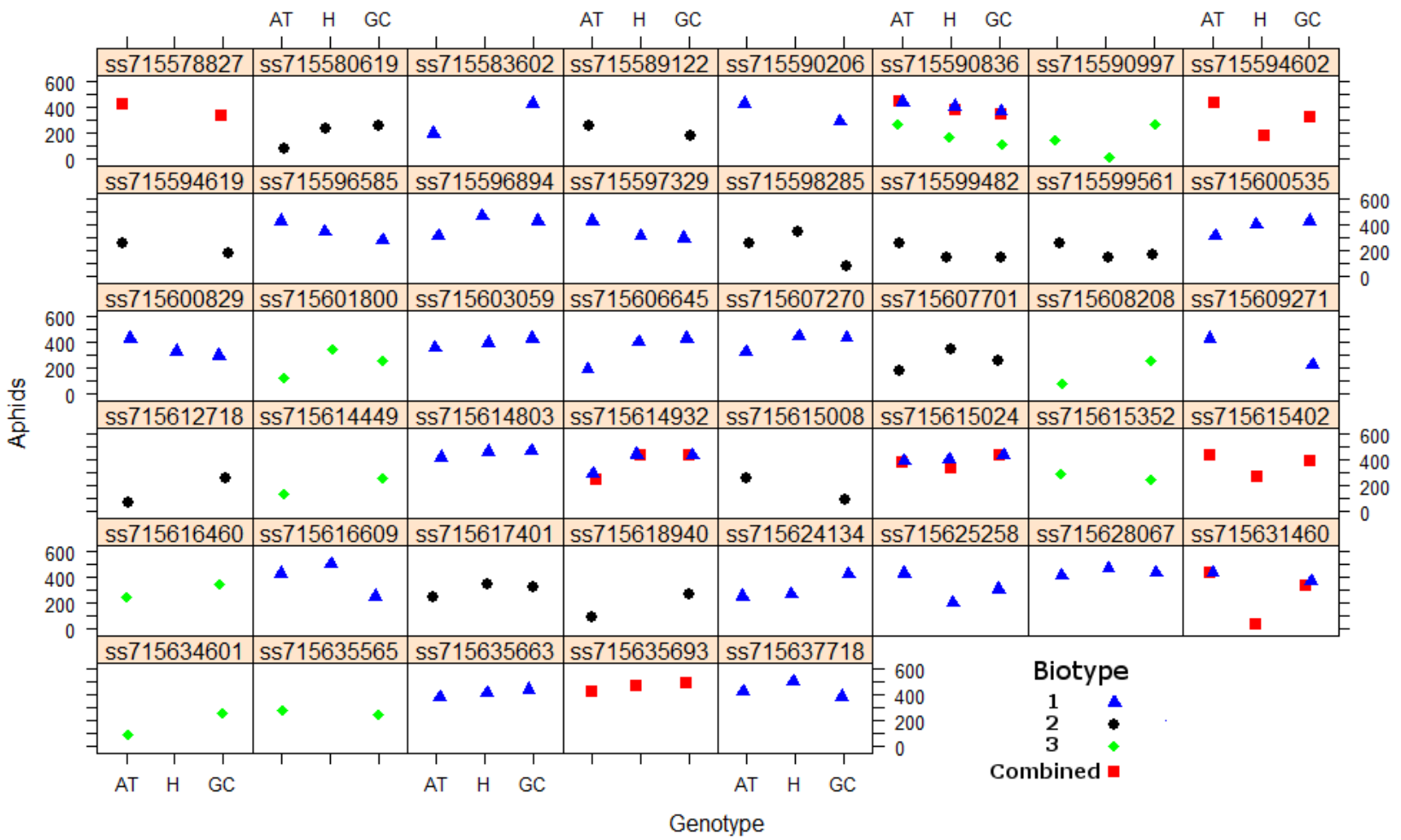

Figure 3.7. Back-transformed least squares means of soybean aphid densities across all studies for homozygous or heterozygous alleles when available at each significant SNP.

Chapter IV: Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid insecticides in the upper Midwestern United States

4.1 Introduction

Soybean, *Glycine max* (L.) Merr., is an important commodity crop in the United States with 33,482,071 ha harvested in 2016 (USDA-NASS, 2016). Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), an invasive insect pest of soybean, is especially prevalent in the Midwest United States and may cause up to 40% yield loss (Ragsdale et al. 2007). Prior to the detection of soybean aphid in North America in 2000, less than 0.1% of soybean fields in the upper Midwest were treated with insecticides. However, in less than 10 years, insecticide use in soybean in response to soybean aphid increased 130-fold and resulted in increased production costs of US$16–$33 per ha (Ragsdale et al. 2007, 2011). During outbreak years, up to 57% of the soybean acres in some states have been treated with foliar insecticides for soybean aphid (Ragsdale et al. 2011). Control costs and yield losses due to soybean aphid amount to US$ 2.4 to 4.9 billion per year (Song et al. 2006; Kim et al. 2008a).

Currently, integrated pest management recommendations for soybean aphid include planting varieties with soybean aphid resistance genes (e.g., *Rag1*), promoting natural enemy populations for pest suppression, and threshold-based applications of insecticides when needed (reviewed by Ragsdale et al. 2011; Hodgson et al., 2012; Hesler et al. 2013). Despite preventative tactics, aphid populations can reach damaging levels where foliar insecticides are required to suppress outbreaks. An economic threshold of 250 aphids per plant was established to prevent aphid populations from reaching the economic injury level of 674 aphids per plant (Ragsdale et al. 2007; Koch et al. 2016).

The adoption of scouting and use of the economic threshold for soybean aphid management is estimated to have produced an economic net benefit of $1.3 billion from 2003 to 2017 (Johnson et al. 2009; Song and Swinton 2009).

Reliance on insecticides for pest management can result in unintended environmental or non-target impacts, such as insecticide resistance, pest resurgence, and pest replacement (Pedigo and Rice 2009). Insecticide resistance occurs when, compared to an unexposed population, an insect population exhibits a genetically-based decrease in susceptibility to a toxin after repeated exposure; resistance can occur on a continuum from low levels of resistance where insecticide applications can still adequately control the pest in the short-term to increased resistance where control failures occur in crop fields (reviewed by Tabashnik et al. 2009, 2014). Genes conferring insecticide resistance may negatively affect other measures of fitness such as number or quality of offspring, but fitness costs are not necessarily always present (reviewed by Kliot Ghanim 2012; ffrench-Constant and Bass 2017). The evolution of insecticide resistance may be slowed by alternating insecticide groups (Sparks and Nauen 2016). However, suppression of soybean aphid outbreaks currently depends on the use of a limited number of broad-spectrum foliar insecticides, primarily pyrethroids and organophosphates (Hodgson et al. 2012; Koch et al. 2016).

Methodologies have been developed for laboratory assessment of soybean aphid resistance to insecticides. Magalhaes et al. (2008) used detached soybean leaves with petioles immersed in different concentrations of neonicotinoids to assess soybean aphid susceptibility. Soybean aphids have also been directly immersed in pyrethroids, organophosphates, and neonicotinoids using an aphid-dip bioassay (Chandrasena et al.

2011). These studies on soybean aphid in North America have not documented insecticide resistance. However, soybean aphid populations in China had "light resistance" to organophosphates and pyrethroids (Wang et al. 2011, 2012). In addition, a Chinese population of soybean aphid was recently confirmed resistant to a pyrethroid, λ-cyhalothrin, after selection for insecticide resistance under laboratory conditions (Xi et al. 2015). That population also exhibited resistance to other pyrethroid (e.g., bifenthrin), organophosphate (e.g., chlorpyrifos) and carbamate (e.g., carbofuran) insecticides (Xi et al. 2015).

After multiple years of insecticide use for soybean aphid management, potential for development of soybean aphid resistance to insecticides poses a threat to soybean production in North America. The purpose of this study was to establish methods for routine monitoring of soybean aphid susceptibility to commonly used pyrethroids, quantify current levels of soybean aphid susceptibility to pyrethroids in the upper Midwest, and monitor for insecticide resistance.

4.2 Materials and Methods

4.2.1 Insects

A laboratory strain of soybean aphid was obtained from the University of Illinois and shipped to Minnesota under United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service permit number P526P-13-00836 to serve as a reference population throughout all experiments. This strain (i.e., biotype 1) collected in 2000, has not been exposed to insecticides since detection in North America (Kim et al. 2008b). The aphids were reared on V3 to V5 growth stage (Fehr and Caviness 1977) SD01-76R soybean plants in environmental growth chambers at 25ºC, 70% relative

humidity and a 16:8 (L:D) h photoperiod (i.e., standard growth chamber conditions used throughout all experiments). New soybean plants were introduced to the colony at least twice per week to ensure consistent plant quality and prevent excess honeydew and sooty mold growth. Aphid infested trifoliates were cut from old plants being removed from the colony and were placed on top of the newly introduced plants to facilitate infestation.

Field populations of soybean aphids were collected from soybean fields in June to September in 2013 to 2016 for use in bioassays (Fig. 4.1; Tables 4.1 and 4.2). Aphids were collected from fields that had not been treated with foliar insecticides in the same year, unless otherwise noted. Aphids were collected from some locations over multiple years, but such collections were from different fields each year. At each location, aphid-infested soybean plants were cut at ground level, cut stems were inserted into wet florist foam, and placed in a plastic 50-liter ice chest with ice packs. Plants were then transported immediately to the laboratory in Saint Paul, Minnesota.

Adult aphids from the field-collected plants were used in bioassays within 24 h of collection, unless otherwise noted. When aphids from a population could not be tested within 24 hours, aphids that were unparasitized and uninfected with entomopathogenic fungi were used to initiate a colony in an environmental growth chamber under the standard rearing conditions described previously until a sufficient number of aphids were available to perform a bioassay. Aphids collected from Dalton in 2013 were placed in colony due to high rates of parasitism and infection by entomopathogenic fungi resulting in low numbers of healthy aphids in the initial collection. Aphids from Lamberton in 2015 and Crookston in 2016, where insecticide applications to fields failed to control

aphid populations, were also maintained in colony due to insufficient numbers of aphids for bioassays in the initial collections.

4.2.2 Leaf-dip bioassays

During 2013 to 2015, a leaf-dip bioassay was used to assess aphid susceptibility to a commercial formulation of λ-cyhalothrin (Warrior II with Zeon Technology®, Syngenta Crop Protection, Inc., Basel, Switzerland). Preliminary bioassays with the laboratory population were used to determine a range of concentrations to use for bioassayed populations (Tables 4.1 and 4.3). After preliminary bioassays, the treatment concentrations used in the leaf-dip bioassay consisted of λ-cyhalothrin prepared at 10% of recommended field rate of 149.5 ppm by pipetting 4.8 µl of insecticide product into 80 ml of reverse osmosis (RO) water. From this highest concentration of 15 ppm, the remaining seven concentrations were created using 25% serial dilutions by the transfer of 20 ml of each previous solution into 60 ml of RO water in order to provide a sufficient amount of solution to submerge the leaf disk. An untreated control of RO water with no insecticide was also included for a total of nine concentrations per independent replication (Table 4.3).

Leaf disks were prepared following methodology of the Insecticide Resistance Action Committee (IRAC) for assessing aphid insecticide resistance (IRAC 2016). First and second trifoliate leaves were collected from uninfested, untreated V5 (Fehr and Caviness 1977) SD01-76R soybean plants grown in growth chambers. Leaf disks were cut from the leaves using a 3.8-cm diameter hole punch (Fiskars, Helsinki, Finland). The leaf disks were manually submerged with gentle agitation in one of the treatment solutions or RO water for 10 seconds and then allowed to air dry abaxial side up on a

paper towel. Dried leaf disks were placed abaxial side up on an agar bed in 29.6 ml soufflé cups (Solo Cup Company, Lake Forest, IL). The agar bed was created from a 1% agar (Fisher Scientific Molecular Genetics Granulated Agar, Thermo Fisher Scientific, Waltham, MA) solution in RO water that was heated to a boil, cooled to 55°C, and then transferred to the plastic soufflé cups. The cups were filled with agar to 10 mm from the top of the cups. Just prior to the agar congealing (71aculat. 35°C), leaf disks were placed on the agar and gently pressed to ensure the entire surface of the leaf disks was in contact with the agar bed. When necessary, a drop of RO water was added to the surface of the agar bed to increase leaf disk adherence.

A fine-tipped camel-hair brush was used to first transfer aphids from source plants to Petri dishes with moistened filter paper. Twenty confirmed uninjured apterous adult aphids from a randomly selected Petri dish were transferred to each disk with fine-tipped brushes to each of the leaf disks in order of increasing insecticide concentration to avoid contaminating leaves with residue from higher concentration treatments. Apterous adult aphids, used for all bioassays, were identified by their extended cauda, dark cornicles and absence of wing pads (Hodgson et al. 2005). The cups were then covered with ventilated lids and placed in a growth chamber at the standard conditions described previously. The leaf-dip bioassays were performed as randomized complete block designs for each population, with three independent replications for each concentration of λ-cyhalothrin or untreated control, except for three population-years. Two independent replications were performed for aphids from Brooten in 2013 due to low numbers of adult aphids. The susceptible laboratory population in 2013 and 2014 was assayed with four and six independent replications, respectively.

Aphid mortality was assessed after 24 and 48 h by visual inspection of the aphids and gentle prodding of the aphids with a fine-tipped camel-hair brush. Dead aphids usually turned a reddish-brown color (Chandrasena et al. 2011). Aphids were defined as dead when they were prodded with the brush and would not move after 10 seconds. Aphids showing a lack of coordinated movement that could not right themselves were considered moribund and counted as dead. These moribund aphids usually had protruding mouthparts that were no longer embedded within the leaf disk and they had very little to no movement when prodded. Aphids that were moribund at 24 hours did not recover at 48 hours (data not shown). In addition, nymphs produced by surviving aphids on each leaf disk were counted and removed at 24 h and counted again at 48 h.

Nymph production of surviving adults was quantified as an additional measure of susceptibility to λ-cyhalothrin. Nymph production was measured as nymphs produced per cumulative adult aphid-day (CAD), based on counts of surviving adult aphids, at each treatment concentration to account for differential adult survival over time. This use of CAD provided a standard measure of both the number of live adult aphids present and the duration of their survivorship, was calculated for each replication using [eq. 1]

$$CAD = \sum_{i=1}^{n} \frac{x_i + x_{i-1}}{2} * t_i \text{ [eq. 1]}$$

where *n* is the number of observation periods (0, 24, and 48 h), *x* is the number of surviving adult aphids per leaf on observation date *t*, and t_i is days since the previous observation date (Ruppel 1983, Hanafi et al., 1989, Kieckhefer et al. 1995). For each replication within a population-year, total nymphs produced over 48 h per CAD at each concentration were divided by nymphs per CAD in the untreated control (i.e., 0 ppm) to account for underlying baseline differences in nymph production among populations

unrelated to bioassay insecticide exposure. This measure of nymph production was used to represent differences in fecundity in response to sublethal insecticide exposure.

4.2.3 Glass-vial bioassays

To develop a more efficient method of assessing susceptibility without plant material, a glass-vial bioassay method was modified from Snodgrass et al. (1996) and Miller et al. (2010). The interior surfaces of 20-ml glass scintillation vials were coated with λ-cyhalothrin or bifenthrin by placing a solution of technical-grade insecticide in acetone into each vial. Vials were placed uncapped on their sides on a hotdog roller (Funtime Popcorn Company®, Ontario, CA, USA) without heat, allowing the acetone to evaporate and evenly coat the interior surface of each vial with insecticide residue. Unlike the leaf-dip bioassays where concentrations were reported as ppm, the glass vials had a known amount of dried active ingredient evenly coating the interior, so concentrations for the glass-vial bioassays are reported as the mass of active ingredient per vial.

In 2015, a stock solution of technical grade λ-cyhalothrin was created with 0.0408 g λ-cyhalothrin (98.0% purity) and 40 ml acetone for a concentration of 0.001 g active ingredient per ml of acetone, and the bifenthrin stock solution was 0.0408 g bifenthrin (97.9% purity) and 40 ml acetone for a concentrations of 1000 μg and 999 μg active ingredient per ml of acetone, respectively. Preliminary bioassays with the laboratory population were used to determine a range of concentrations to use for bioassayed populations (Tables 4.2 and 4.3). For each active ingredient, concentrations used for bioassays consisted of a 0.39% dilution from to stock solution for λ-cyhalothrin and 1.17% dilution from stock solution for bifenthrin followed by nine 25% serial dilutions

for each insecticide, and the evaporated acetone control (11 total concentrations per replication per active ingredient) (Table 4.3). For treatment of vials, 1 ml of each solution was pipetted into its respective treatment vials.

In 2016, a stock solution of technical grade λ-cyhalothrin was created with 0.0141 g λ-cyhalothrin (91.1% purity) and 69.876 ml acetone for a concentration of 184 g active ingredient per ml of acetone, and the bifenthrin stock solution was 0.0131 g bifenthrin (97.9% purity) and 64.854 ml acetone for a concentration of 198 μg active ingredient per ml of acetone. Stock solutions were kept in a -20°C freezer between bioassays. Preliminary bioassays were used to determine a range of concentrations for assaying populations (Table 4.2). For λ-cyhalothrin, concentrations used for bioassays consisted of three 25% serial dilutions from the stock solution followed by eight 40% serial dilutions, and the evaporated acetone control (12 total concentrations per replication) (Table 4.3). For bifenthrin, concentrations used for bioassays consisted of three 25% serial dilutions followed by ten 40% serial dilutions from the stock solution, and the evaporated acetone control (14 total concentrations per replication) (Table 4.3). For treatment of vials, 0.5 ml of each solution was pipetted into its respective treatment vial.

Similar to the leaf-dip bioassay, aphids were transferred from soybean plants to a petri dish with moistened filter paper to confirm aphids were uninjured, apterous adults. Ten apterous adult aphids were then transferred with fine-tipped brushes to the bottoms of the treated vials. The vials were capped and held upright in a growth chamber at the standard conditions described above. The glass-vial bioassays were performed as randomized complete block designs for each population and insecticide with three independent replications of each concentration or acetone control in glass-vials for λ-

cyhalothrin and bifenthrin for most populations. Six replications were performed throughout the growing season in 2015 with λ-cyhalothrin using the laboratory population. Nine and eight replications were also used for λ-cyhalothrin and bifenthrin, respectively, in 2016 bioassays using the laboratory population.

Aphid mortality was assessed after 4 and 24 h by visual inspection of the aphids in the vials. Aphids that were lacking coordinated movement, unable to right themselves, or unable climb the walls of the vial after 10 seconds of the vial being turned on its side were considered moribund and counted as dead. Aphids showing no movement were considered dead. Live aphids typically would be found walking on the walls of the vials, whereas moribund or dead aphids fell to the bottoms of the vials.

4.2.4 Data analysis

Mortality data were analyzed using SAS 9.4. For leaf-dip and glass-vial bioassays, analyses were performed on mortality data from 48 and 4 h, respectively. Overall differences in mortality as a function of population and concentration were assessed using ANOVA (PROC GLIMMIX). Proportion mortality was analyzed with a log-logistic function [eq. 2]

$$\ln(m) = -e^{-(i+sx)} \quad \text{[eq. 2]}$$

where *m* is mortality, *I* represents the model intercept, *s* is the slope, and *x* is the insecticide concentration. The log-logistic distribution was used instead of a logistic distribution due to improved model fit. Prior to analysis, a modified Abbot correction (Rosenheim and Hoy, 1989) was used to account for control mortality in each replication by producing an adjusted number of survivors and total sample size (e.g., Hanson et al. 2013). Insecticide concentrations were transformed with a natural-log(concentration+1) transformation to satisfy the assumption of normal distribution of residuals while

improving model fit. A residual term was also used for the leaf-dip and glass-vial bioassays to account for over-dispersion.

Model terms included a continuous effect (i.e., slope) of insecticide concentration and intercepts for population, as well as intercepts year for leaf-dip bioassays. Interactions of concentration with population or year were also included. Nested replication and replication by concentration effects within each population-year and population were included for leaf-dip and glass-vial bioassays (i.e., each set of serial dilutions for each replication was independently created from the stock solution for each population-year). Because of significant population and insecticide concentration interactions, an analysis of covariance (ANCOVA) alone was not appropriate as differences among populations would change depending on insecticide concentration (e.g., Hardman et al. 2000). To capture these differences due to insecticide concentrations, we first performed statistical comparisons among populations at the average of the concentrations using a one-tailed Dunnett's test that provides formal probability values for multiple comparisons to determine if field populations had decreased susceptibility at that concentration compared to the reference laboratory population. Because of the insecticide concentration and population interactions, differences among populations at other points on the dose-response curves were assessed. We determined concentrations causing 50% (i.e., LC_{50}) and 90% (i.e., LC_{90}) mortality and their confidence intervals to compare single point estimates of mortality by non-overlapping confidence intervals calculated from the dose-response models (Faraggi et al. 2003).

Nymph production measures in the leaf-dip bioassays were first natural-log+1 transformed and then fit to an inverse distribution to fulfill normality and homogeneity of

variance assumptions. The transformed data were analyzed using an ANOVA (PROC GLIMMIX). Similar to the mortality analyses, effective concentrations were calculated resulting in 50% (EC_{50}) and 90% (EC_{90}) reductions in nymphs produced per CAD based on surviving adult aphids.

4.3 Results

4.3.1 Leaf-dip bioassays

Overall control mortality at 48 hours across populations averaged approximately 13% and ranged from 0 to 37% per replication. Higher control mortality was typically found in populations with moderate parasitism or fungal infections. The mortality of aphid populations from λ-cyhalothrin measured at 48 h varied across populations and years sampled, which was indicated by significant population and year effects (i.e., intercept), concentration effect (i.e., slope), and interactions of population by concentration and year by concentration (Table 4.4). In 2013, Becker, Dalton, and Lamberton populations had significantly decreased λ-cyhalothrin-induced mortality (*Dunnett's* $t \geq 2.45$, $df = 483$, $p \leq 0.038$) at 0.796 ppm, the average of tested concentrations back-transformed from the natural-log, compared to the laboratory population; LC_{50}s for Dalton and Lamberton, and the LC_{90} for Lamberton were also significantly higher than the laboratory population (Fig. 4.2a). In 2014, none of the field-collected populations had significantly less λ-cyhalothrin-induced mortality than the laboratory population (Fig. 4.2c). In 2015, significantly decreased λ-cyhalothrin-induced mortality at 0.796 ppm was found in a Lamberton population collected on 5 Aug (*Dunnett's* $t = 3.22$, $df = 483$, $p = 0.003$) where a field application of bifenthrin failed to control aphid populations prior to collection; the LC_{50} and LC_{90} for Lamberton collection

on 5 Aug and the LC_{50} for Becker were also significantly higher than the laboratory population (Fig. 4.2e).

The proportional change in nymph production at sub-lethal concentrations after adjusting for each population's baseline nymph production in unexposed control treatments varied across populations and years, which was indicated significant population and year effects (i.e., intercept), concentration (i.e., slope), and interactions of population by concentration and year by concentration (Table 4.4). Aphids from Dalton, Becker, and Lamberton in 2013 (*Dunnett's* $t \geq 4.09$, $df = 483$, $p < 0.001$), as well as Lamberton, St. Paul, and Brooten in 2014 (*Dunnett's* $t \geq 3.52$, $df = 483$, $p < 0.001$) experienced less suppression of reproduction at 0.796 ppm, the back-transformed average of λ-cyhalothrin concentrations tested, compared to the laboratory population; these populations also had significantly higher EC_{50}s and EC_{90}s than the laboratory population within each year (Fig. 4.3 a & c). In 2015, aphids from St. Paul, Lamberton collected on 20 July, Becker, and Lamberton collected on 5 August after a bifenthrin application in the field failed to control the aphid population experienced less overall suppression of reproduction at 0.796 ppm than the laboratory population (*Dunnett's* $t \geq 2.90$, $df = 483$, $p \leq 0.006$); these populations also had significantly higher EC_{50}s and EC_{90}s than the laboratory population (Fig. 4.3e & f). Both LC and EC values across years were positively correlated at 50% and 90% values with approximately a 4-fold increase in EC values compared to LC values (Fig. 4.4); this indicated that populations that had lower mortality from λ-cyhalothrin exposure also tended to require higher concentrations of insecticide to suppress reproduction of surviving adults.

4.3.2 Glass-vial bioassays

In glass-vial bioassays, overall control mortality at 4 h across populations averaged approximately 1% and ranged from 0 to 10% per replication. Assessments at 24 h were not included because overall control mortality varied greatly, ranging between 0 to 100% mortality. In the 2015 λ-cyhalothrin bioassays with the laboratory and Lamberton populations, there was not a significant population effect (i.e., intercept), but there was a significant concentration effect (i.e., slope) and population by concentration interaction indicating differences in mortality among populations were dependent upon insecticide concentration (Table 4.5). The Lamberton population also had significantly lower λ-cyhalothrin-induced mortality at 0.263 μg, the back-transformed average of tested λ-cyhalothrin concentrations back-transformed from the natural-log (*Dunnett's t* = 4.66, *df* = 81, $p < 0.001$), and the LC_{50} and LC_{90} were significantly higher than the laboratory population (Fig. 4.5a). For bifenthrin in 2015, there also was not a significant population effect (i.e., intercept), but there was a significant concentration effect (i.e., slope) and population by concentration interaction (Table 4.5). The Lamberton population also had significantly lower bifenthrin-induced mortality at 0.531 μg, the back-transformed average of tested bifenthrin concentrations (*Dunnett's t* = 4.37, *df* = 54, $p < 0.001$), and the LC_{50} and LC_{90} were also significantly higher than the laboratory population (Fig. 4.6a).

In 2016, aphid mortality varied across populations for both λ-cyhalothrin and bifenthrin. For λ-cyhalothrin, population effect (i.e., intercept), concentration effect (i.e., slope), and the population by concentration interaction were significant (Table 4.5). Aphids from Lamberton, Chandler, Calumet, Windom, and Crookston each had significantly lower λ-cyhalothrin mortality at 0.760 μg, the back-transformed average of

tested λ-cyhalothrin concentrations, than the laboratory reference population (*Dunnett's t* ≥ 5.65, *df* = 291, $p < 0.001$) ; excluding Lamberton, these populations also had significantly higher LC_{50}s and LC_{90}s than the laboratory population (Fig. 4.5c). For bifenthrin, the population effect (i.e., intercept) was not significant, but concentration effect (i.e., slope) and population by concentration interaction were significant (Table 4.5). Aphids from Chandler, Crookston, Lamberton (2), Calumet, Windom, and Lamberton (1) each had significantly lower bifenthrin mortality (*Dunnett's t* ≥ 5.13, *df* = 312, $p < 0.001$) at 0.650 μg, back-transformed the average of tested bifenthrin concentrations, than the laboratory reference population; excluding Lamberton (2) and Windom, these populations also had significantly higher LC_{50}s and LC_{90}s than the laboratory population (Fig. 4.6c).

4.4 Discussion

Here we provide the first evidence for soybean aphid resistance to insecticides in North America. Documentation of resistance by a significant decrease in insecticide susceptibility with laboratory bioassays in 2015 and 2016 coincided with numerous reports over a large geographic area of failures of field applications of pyrethroids (primarily bifenthrin, bifenthrin plus zeta-cypermethrin, and λ-cyhalothrin) to control soybean aphid. In 2015, pyrethroid failures were reported from Martin, Faribault, Blue Earth, Cottonwood, Redwood, Brown, Renville, Dakota and Sherburne counties in Minnesota (Potter, Koch and MacRae, *unpublished data*). In 2016, pyrethroid failures were reported from Redwood, Murray, Cottonwood, Jackson, Martin, Watonwan, and Polk counties in Minnesota and O'Brien County in Iowa (Potter, Koch, MacRae and Hodgson, *unpublished data*). Visits to several fields with poor pyrethroid efficacy

revealed multiple application methods, application dates, and pyrethroid products were involved. Often, pockets of minimal aphid control interspersed with good control were observed suggesting within-field genetic clonal differences. Results of laboratory bioassays presented here indicate that aphids in some of these situations had significant levels of resistance to pyrethroids.

Using the leaf-dip bioassays, we characterized susceptibility to λ-cyhalothrin of soybean aphid from Minnesota fields from 2013 to 2015 that can be used as a reference level of susceptibility for future years. In 2013 and 2015, populations were found that required approximately 2.1- to 3.9-fold more λ-cyhalothrin than needed to cause the same amount of mortality in the laboratory population (Fig. 4.2b & f). These LC_{50}s from 0.94 to 1.71 ppm were still well below the recommended field rate of 149.5 ppm for λ-cyhalothrin in Warrior II, though actual concentrations experienced on the plant could be affected by factors such as plant canopy and weather (Guillebeau et al. 1989). The highest LC_{90} of 12.6 (95% CL: 5.03 – 29.9) ppm from the Lamberton 2013 population, or nine-fold increased resistance compared to the laboratory population, was approximately 8% of the recommended field rate.

Chandrasena et al. (2011) also performed bioassays on susceptible soybean aphid by dipping groups of aphids in a solution λ-cyhalothrin using a tea strainer. Their LC_{50} of 0.054 ppm for the aphid-dip bioassay is likely lower compared to our leaf-dip bioassay because aphids in that bioassay were directly submerged in the insecticide solution instead of being exposed via residual contact on treated leaves (Chandrasena et al. 2011). In other bioassays using λ-cyhalothrin on aphid species, LC_{50}s have ranged from 0.26 ppm for susceptible *A. gossypii* to 53 to188 ppm for resistant populations in leaf-dip

bioassays (Ahmad et al. 2003). Λ-cyhalothrin-susceptible *Brevicoryne brassicae* collected in 2006 had an LC_{50} of 0.37 ppm and increased to 1.63 ppm in 2007 and 91.4 ppm in 2010 (Ahmad and Akhtar 2013). Our LC_{50}s in λ-cyhalothrin leaf-dip bioassays for laboratory populations (0.32 to 0.44 ppm) and the most resistant field population (1.71 ppm), approximately matched Ahmad and Akhtar's (2013) *B. brassicae* initial baseline population and the resistant population the following year, respectively.

Variation in insecticide mortality over time was also documented. None of the locations from which aphids were tested using the leaf-dip bioassay had decreased mortality in 2014, although these locations had decreased mortality in 2013 and 2015. Aphids collected from Lamberton in Aug 2015 came from a location where a field application of bifenthrin failed to control the aphid population. Although this population exhibited a 4-fold decrease in mortality, aphids collected in earlier July from a nearby field (82aculat. 1.5 km) before insecticides were applied did not exhibit decreased mortality (Fig. 4.2). Genetic heterogeneity could explain these differences. Local population variation due to founder effects within the field or among nearby fields could explain field-level variation (Orantes et al. 2012). Mortality could also vary regionally throughout the year as soybean aphids migrating from surrounding fields or other regions increase genotypic diversity as the season progresses (Michel et al. 2009; Orantes et al. 2012; Schmidt et al. 2012; Bahlai et al. 2014). The frequency of resistant genotypes in individual fields may also be lower earlier in the growing season and increase regionally as foliar insecticides are applied later in summer, which could complicate efforts to detect resistance early in the growing season. Mortality in bioassays can also vary depending on aphid age. Early-instar soybean aphid nymphs are, for instance, the most susceptible to

neonicotinoids, but mortality and population growth after neonicotinoid exposure does not significantly vary between mixed-age aphids and age-synchronized adults (Ribeiro 2017).

Suppression of nymph production (i.e., EC_{50} and EC_{90}) in leaf-dip bioassays appears to be an additional effective measure for assessing soybean aphid susceptibility to λ-cyhalothrin. Generally, concentrations of λ-cyhalothrin required to suppress nymph production by surviving aphids were relatively high in populations with lower insecticide-induced mortality (Fig. 4.4). For some populations, such as those from several locations in 2014 and Lamberton in 2015, EC_{50}s could be used to detect resistance to the insecticide that was not detected based on LC_{50}s. Since the EC_{50} and EC_{90} values are standardized to account for nymph production by surviving adults on untreated leaves, these values indicate that adults from some populations experience less of a reduction in reproductive output after exposure to sub-lethal concentrations of λ-cyhalothrin. When a population has both decreased mortality and decreased suppression of nymph production after exposure to insecticide, such as Lamberton in 2013 with over a 50-fold higher EC_{50}, it is concerning that potentially resistant adult aphids are not only surviving more, but also maintaining reproductive capacity. This capacity to maintain reproductive rate may indicate there is not a fitness cost associated with decreased mortality from insecticide exposure. Sub-lethal concentrations of beta-cypermethrin, another pyrethroid, can cause hormesis, or increased nymph production, in soybean aphids (Qu et al. 2017). While EC measures do not always correlate with decreased mortality, such populations may maintain higher net reproductive rates than susceptible control aphids (Qu et al. 2015).

The glass-vial bioassays also documented resistance to λ-cyhalothrin and bifenthrin in multiple soybean aphid populations. In the 2015 glass-vial bioassays, the Lamberton population collected on Aug 24 had 10- and 38-fold resistance to λ-cyhalothrin and bifenthrin, respectively, compared to the susceptible laboratory population (Figs. 4.5b & 4.6b). In 2016, the Lamberton population collected in June on volunteer soybean had approximately 10-fold resistance to bifenthrin, which indicates that some early-season aphids already had some degree of resistance prior to any foliar insecticide applications for soybean aphid that year (Fig. 4.6b and c). Each of the populations collected from fields with pyrethroid control failures in 2016 had statistically significant levels of resistance to one or both pyrethroids, which indicates that failures in some fields were likely due to resistance, as opposed to other factors such as applicator error or environmental conditions (Guillebeau et al. 1989).

Not all aphid populations showed resistance to both pyrethroids. For example, the Lamberton population in 2015 had resistance to both λ-cyhalothrin and bifenthrin, but the Lamberton population collected in Aug 2016 and adjacent to a bifenthrin failure was only resistant to bifenthrin. Cross resistance between λ-cyhalothrin and bifenthrin has been previously documented. Using a leaf-dip bioassay, Xi et al. (2015) found cross resistance in a laboratory-selected population of soybean aphid from China with 77-fold resistance to λ-cyhalothrin and 4.8-fold resistance to bifenthrin compared to a susceptible aphid colony. Soybean aphid can become 40-fold resistant compared to susceptible aphids after 25 generations of pyrethroid exposure under laboratory conditions (Bi et al. 2016). We documented populations at or near 40-fold resistance in this study, which indicates Midwest soybean aphids have likely undergone multiple generations of selection pressure

for pyrethroid resistance. There are also cases such the leaf-dip bioassay with aphids from Lamberton in 2013 where the LC_{90} resistance ratio was much higher than the LC_{50} resistance ratio (Fig. 4.2b). This effect, evidenced by the significant by population by concentration interactions (Table 4.4), could indicate that even stronger resistance exists within a small subset of these populations. Increases in pyrethroid resistance frequency are likely expedited by soybean aphid generation times of 8 to 13 days between 20 and 30°C (McCornack et al. 2004).

The leaf-dip and glass-vial bioassay methodologies have various strengths and weaknesses. The leaf-dip bioassay required more effort, particularly for maintaining plants and preparing treated leaf disks, but it can provide information on both mortality and reproduction. Leaf-dip bioassays generally required at least 24 person-hours per location or 8 person-hours per replication to prepare chemical treatments, transfer adult aphids, and assess aphid mortality. The glass-vial bioassays took less time and effort to prepare with 10.5 person-hours per location or 3.5 person-hours per replication for vial preparation, adult aphid transfer and mortality assessments. Therefore, the glass-vial methodology required about 2.3 times fewer person-hours than leaf-dip bioassays. Treated vials can be easily shipped to collaborators for more geographically dispersed monitoring programs.

Differences in leaf-dip and glass-vial methodologies and potential effects on mortality prevent direct comparisons of leaf-dip and glass-vial bioassays. For example, there was a known amount of active ingredient spread throughout the interior surface of the glass-vials. However, the amount of insecticide remaining on leaf disks was unknown after submerging them in known concentrations. Conversely, no plant material was

present in the glass-vials, which could cause the aphids to become stressed due to lack of food and also increase their movement within vials, thereby increasing their exposure to insecticide. Likely due to the lack of plant material, the 24 h glass-vial assessment of mortality after exposure was not efficient due to highly variable control mortality. However, the 4 h glass-vial assessment, which is a similar timeframe used for other hemipteran glass-vial bioassays (Prabhaker et al. 1996; Hollingsworth 1997; Willrich et al. 2003), never exceeded 10% control mortality.

Resistance of aphids to pyrethroids can occur through multiple mechanisms, such as increased detoxification through upregulation of cytochrome P450-related genes, decreased binding of pyrethroids to the target sites through mutations to sodium channels, and decreased exposure through decreased cuticular penetration (Liu 2012). Additionally, increased production of carboxylesterase has led to resistance of *Myzus persicae* Sulzer (Hemiptera: Aphididae) to pyrethroids, organophosphates and carbamates (Foster et al. 2007). For soybean aphid in China, Xi et al. (2015) found upregulation of cytochrome P450 was associated with resistance to pyrethroid and organophosphate insecticides. In addition, Bi et al. (2016) determined that resistance of soybean aphid to pyrethroids in China was associated with proteins affecting the cytoskeleton (e.g., microtubules, actin, and the cuticle), glycolysis, amino acid synthesis, protein folding, and detoxification metabolism. Further research is needed to examine mechanisms for soybean aphid resistance to pyrethroids in North America.

Management of soybean aphid in soybean continues to rely primarily on foliar application of relatively few insecticide groups (Hodgson et al. 2012). Such reliance on insecticides increases risk for development of insecticide resistance in the target pest

(Pedigo and Rice 2009). The presence of insecticide resistance in soybean aphid populations in North America shows that further education on and implementation of integrated pest management and insecticide resistance management is required to decrease selection pressure for resistance development. Use of scouting and research-based economic thresholds to guide application of foliar insecticides (Hodgson et al. 2012; Koch et al. 2016) will decrease unnecessary insecticide inputs that can contribute to selection for insecticide resistance (Tabashnik 1990; Hoy 1998; Bielza 2008). Insecticides representing groups other than those of the pyrethroids and organophosphates are needed to improve insecticide rotations for this pest, particularly if the insecticides are more compatible with biological control (Pezzini and Koch 2015; Tran and Koch 2016). Increased use of foliar formulations of neonicotinoid insecticides is complicated by soybean aphids being exposed to widespread use of this insecticide group as seed treatments (Douglas and Tooker 2015). Greater adoption of other non-chemical tactics for soybean aphid management, such as host-plant resistance (Hesler et al. 2012; Hanson et al. 2016b) will further decrease selection pressure for insecticide resistance.

Increased monitoring for soybean aphid resistance to pyrethroids is needed to better characterize the scope of this problem and to alert growers when pyrethroids may not be a feasible control option for this pest. Diagnostic concentrations calculated from our data, such as LC_{90}s from leaf-dip or glass-vial bioassays with the laboratory population, can be used to assay field populations (Mascarenhas and Boethel 2000; Jin et al. 2015). Such diagnostic concentrations could quickly determine if aphids from a field with a suspected insecticide failure are indeed resistant and warrant a more thorough

dose-response characterization. In order to further advise growers, diagnostic concentrations from our findings could also be used to proactively monitor regionally on an annual basis for soybean aphid resistance prior to insecticide application (Foster et al. 2007).

4.5 Acknowledgements

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4.6 Tables

Table 4.1. Soybean aphid collection locations and dates in Minnesota for leaf-dip bioassays from 2013 to 2015.

Year	Location	Collection	Bioassay date
2013	Laboratory	-	9 July – 13 Aug, 2 Dec – Jan 10
	St. Paul	21-Jul	22-Jul
	Rochester	25-Jul	26-Jul
	Becker	30-Jul	31-Jul
	Lamberton	7-Aug	8-Aug
	Brooten	18-Aug	19-Aug
	Rosemount	25-Aug	26-Aug
	Dalton[1]	4-Sep	2 Dec – 10 Jan
2014	Laboratory	-	23 Jun – 15 July
	Rosemount	20-Jul	21-Jul
	Rochester	28-Jul	29-Jul
	Becker	30-Jul	31-Jul
	Lamberton	5-Aug	6-Aug
	Brooten	11-Aug	12-Aug
	St. Paul	18-Aug	19-Aug
2015	Laboratory	-	30-Jun
	St. Paul	13-Jul	14-Jul
	Lamberton	20-Jul	21-Jul
	Becker	23-Jul	24-Jul
	Brooten	3-Aug	4-Aug
	Lamberton [2]	5-Aug	6-Aug
	Rochester	10-Aug	11-Aug

Location indicates either the unexposed laboratory population or the closest city to the field collection site. Aphids that were not assayed the day after collection were kept in growth chambers in conditions similar to the laboratory population until they were assayed.

[1] Aphids collected after a field application of λ-cyhalothrin failed to control soybean aphid populations.

[2] Aphids collected after a field application of bifenthrin failed to control soybean aphid populations.

Table 4.2. Soybean aphid collection locations and dates in Minnesota and Iowa for glass-vial bioassays from 2015 and 2016 glass-vial bioassays.

Year	Location	Collection date	λ-cyhalothrin bioassay	Bifenthrin bioassay
2015	Laboratory	-	2 – 16 Oct	5-Oct
	Lamberton[1]	24-Aug	26-Oct	19-Oct
2016	Laboratory	-	6 July – 13 Oct	28 July – 27 Oct
	Lamberton (1)[2]	14-Jun	-	15-Jun
	Crookston[3]	18-Jul	15-Sep	22-Sep
	Chandler	19-Jul	20-Jul	20-Jul
	Lamberton (2)[4]	8-Aug	9-Aug	9-Aug
	Rochester	16-Aug	17-Aug	17-Aug
	Windom[1]	23-Aug	24-Aug	24-Aug
	Calumet, IA[1]	23-Aug	13-Oct	27-Oct

Location indicates either the unexposed laboratory population or the closest city to the field collection site. Aphids that were not assayed the day after collection were kept in growth chambers in conditions similar to the laboratory population until they were assayed.

[1] Aphids collected after a field application of bifenthrin failed to control soybean aphid populations. Lamberton aphids in 2015 were collected from the same field as the second collection of Lamberton aphids in the 2015 leaf-dip bioassay (Table 4.1)

[2] Collected from volunteer soybean.

[3] Aphids collected after a field application of λ-cyhalothrin failed to control soybean aphid populations.

[4] Untreated field adjacent to a field where bifenthrin failed to control soybean aphid populations.

Table 4.3. Concentrations of insecticides used in soybean aphid leaf-dip and glass-vial bioassays in addition to control treatments.

Leaf-dip (ppm)	Glass vial (µg/vial)			
λ –cyhalothrin	λ –cyhalothrin		Bifenthrin	
2013-2015	2015	2016	2015	2016
15	3.9	23	11.7	24.7
3.7	0.975	5.74	2.92	6.18
0.93	0.244	1.44	0.731	1.54
0.23	0.061	0.574	0.183	0.618
0.058	0.0152	0.23	0.0457	0.247
0.015	0.00381	0.0919	0.0114	0.0989
0.0036	0.000952	0.0368	0.00285	0.0395
0.00091	0.000238	0.0147	0.000714	0.0158
	0.0000595	0.00588	0.000178	0.00633
	0.0000149	0.00235	0.0000446	0.00253
		0.000941		0.00101
				0.000405
				0.000162

Table 4.4. Regression results for mortality and nymph production of Minnesota populations of soybean aphid in leaf-dip bioassays from 2013 to 2015.

Response	Effect	*DF*	*F*	*p*
Mortality	Concentration	1, 483	96.8	<0.001
	Population	8, 483	2.4	0.010
	Year	2, 483	1.6	0.201
	Concentration*Population	8, 483	5.3	<0.001
	Concentration*Year	2, 483	8.5	0.002
	Population*Year	11, 483	1.9	0.040
	Concentration*Population*Year	11, 483	4.3	<0.001
	Replication(Population*Year)	47, 483	0.7	0.950
	Replication*Concentration(Population*Year)	47, 483	1.1	0.299
Nymph production				
	Concentration	1, 483	168.6	<0.001
	Population	8, 483	2.7	0.006
	Year	2, 483	0.1	0.893
	Concentration*Population	8, 483	13.0	<0.001
	Concentration*Year	2, 483	8.0	<0.001
	Population*Year	11, 483	3.4	<0.001
	Concentration*Population*Year	11, 483	4.5	<0.001
	Replication(Population*Year)	47, 483	2.6	<0.001
	Replication*Concentration(Population*Year)	47, 483	2.3	<0.001

Table 4.5. Regression results for mortality of Minnesota and Iowa populations of soybean aphid in glass-vial bioassays from 2015 and 2016.

		2015			2016		
Insecticide	Effect	*DF*	*F*	*p*	*DF*	*F*	*p*
Bifenthrin	Concentration	1, 54	9.7	0.003	1, 312	138.1	<0.001
	Population	1, 54	<0.1	0.971	7, 312	5.5	<0.001
	Concentration*Population	1, 54	8.2	0.006	7, 312	12.8	<0.001
	Replication(Population)	4, 54	1.4	0.238	16, 312	0.4	0.989
	Concentration*Replication (Population)	4, 54	0.8	0.522	16, 312	1.1	0.358
λ – cyhalothrin	Concentration	1, 81	26.7	<0.001	1, 264	264.8	<0.001
	Population	1, 81	2.7	0.102	6, 264	6.1	<0.001
	Concentration*Population	1, 81	21.4	<0.001	6, 264	37.1	<0.001
	Replication(Population)	7, 81	2.4	0.028	14, 264	2.5	0.002
	Concentration*Replication (Population)	6, 81	1.5	0.189	14, 264	1.6	0.073

4.7 Figures

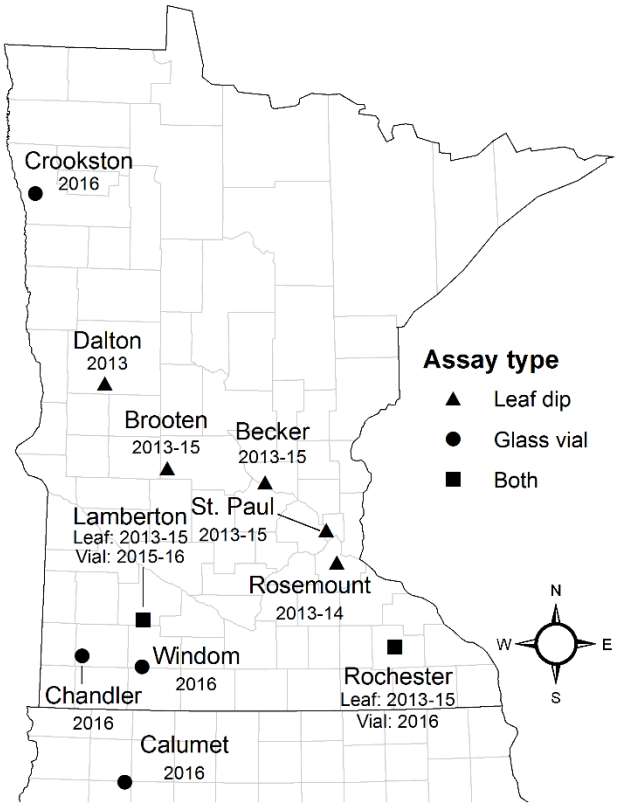

Figure 4.1. Minnesota and Iowa soybean aphid collection locations for multi-year leaf-dip and glass-vial bioassays.

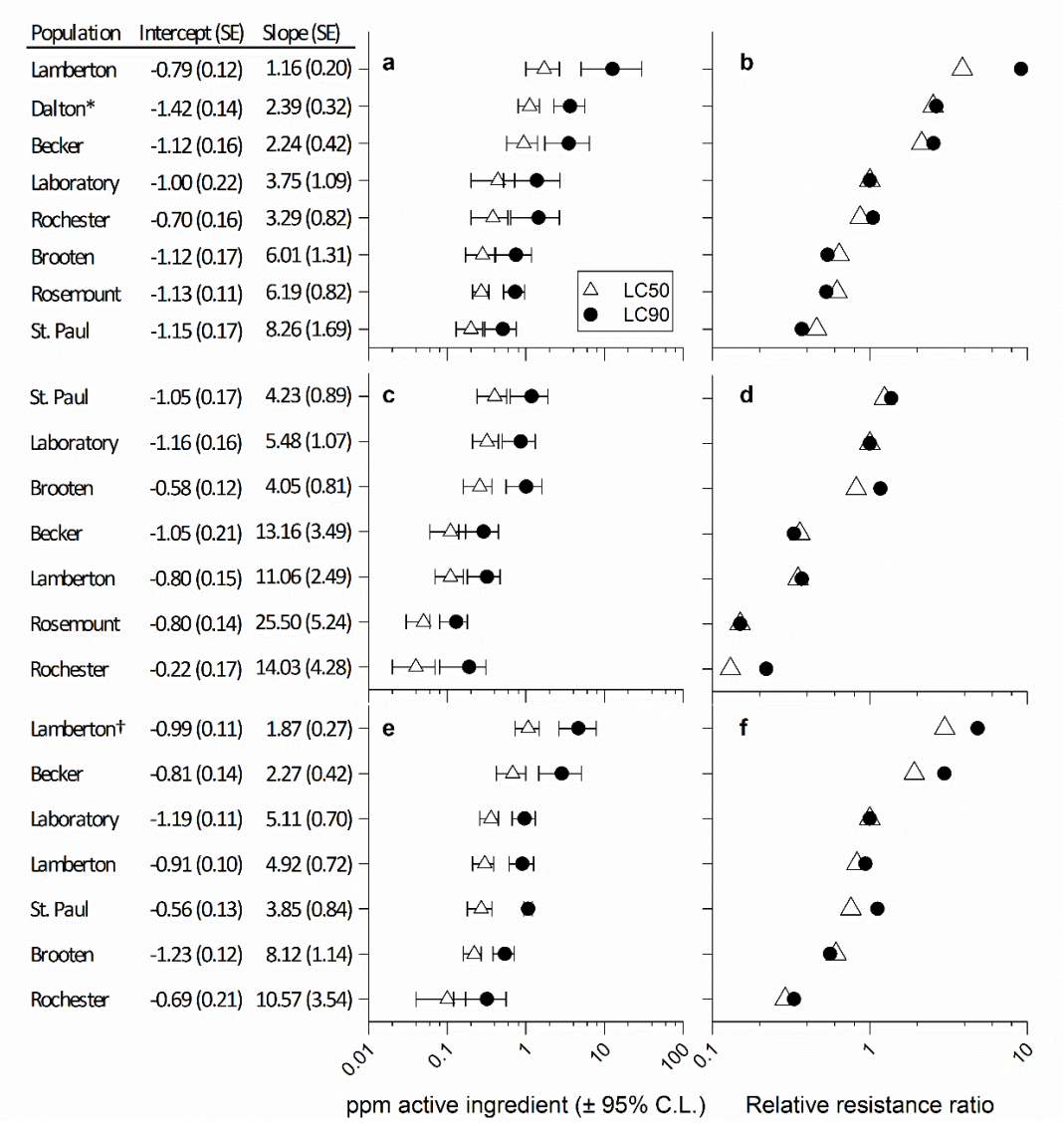

Figure 4.2. Concentrations (ppm) causing 50 and 90% mortality (**a, c &e**) and relative resistance (**b, d & f**) of Minnesota soybean aphid populations in λ-cyhalothrin leaf-dip bioassays for 2013 (**a** & **b**), 2014 (**c** & **d**), and 2015 (**e** & **f**). Relative resistance ratio is the LC_{50} (lethal concentration at 50% mortality) or LC_{90} for a population divided by the corresponding LC of the laboratory population within a year. Concentrations were natural-log back-transformed. An asterisk (*) or dagger (†) indicates aphids collected after an application of λ-cyhalothrin or bifenthrin, respectively, failed to control aphid populations. Confidence intervals that do not overlap within LC_{50} or LC_{90}s indicate estimates for the populations are significantly different.

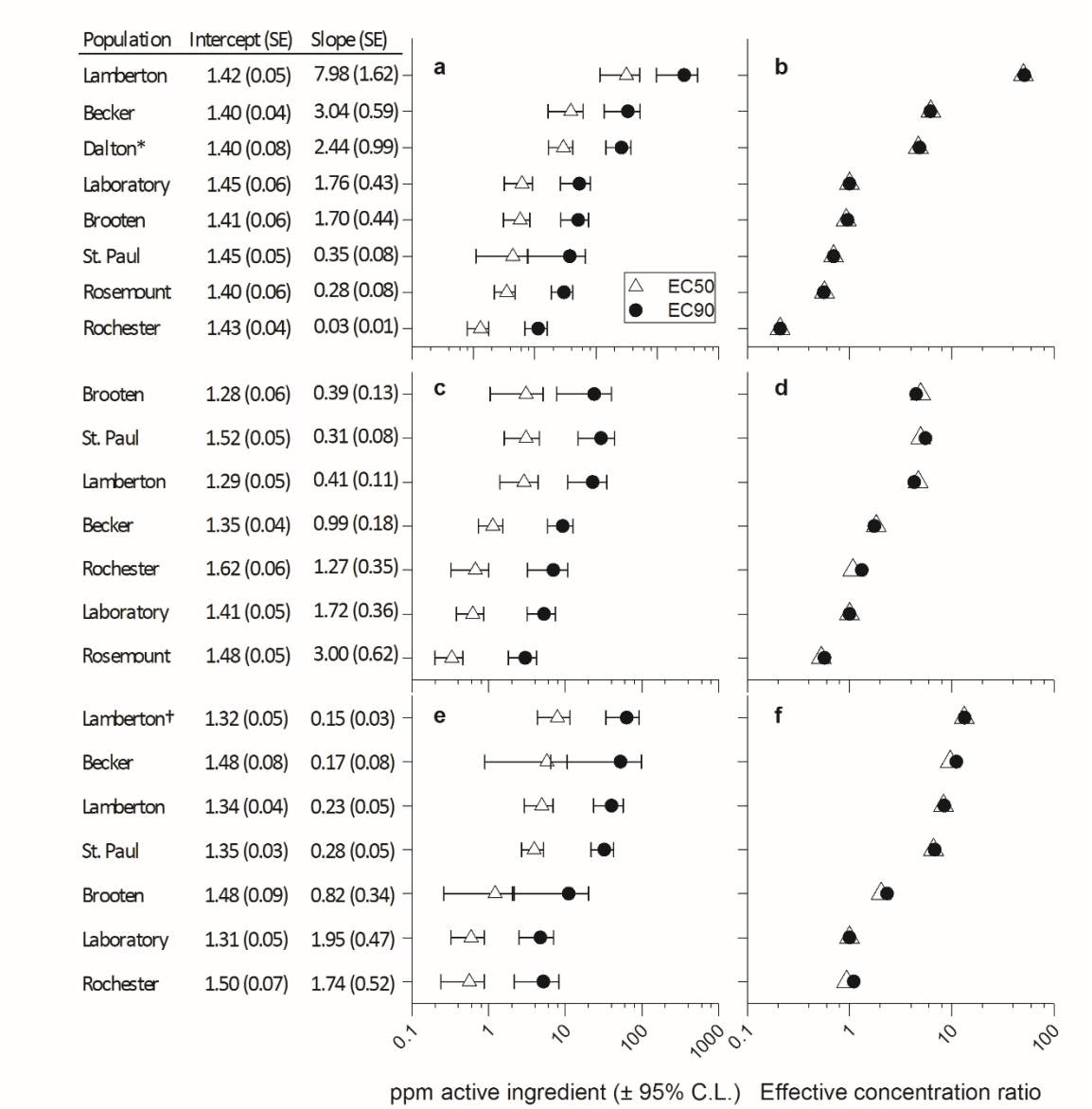

Figure 4.3. Concentrations (ppm) causing 50 and 90% reductions in nymph production by surviving adult soybean aphids (**a, c &e**) and effective concentration ratios (**b, d & f**) of Minnesota soybean aphid populations in λ-cyhalothrin leaf-dip bioassays for 2013 (**a** & **b**), 2014 (**c** & **d**), and 2015 (**e** & **f**). Effective concentration (EC) ratio is the EC_{50} (concentration needed to reduce nymph production by 50% in surviving aphids) or EC_{90} for a population divided by the corresponding EC of the laboratory population within a year. An asterisk (*) or dagger (†) indicates aphids collected after an application of λ-cyhalothrin or bifenthrin, respectively, failed to control aphid populations. Confidence intervals that do not overlap within EC_{50} or EC_{90}s indicate estimates for the populations are significantly different.

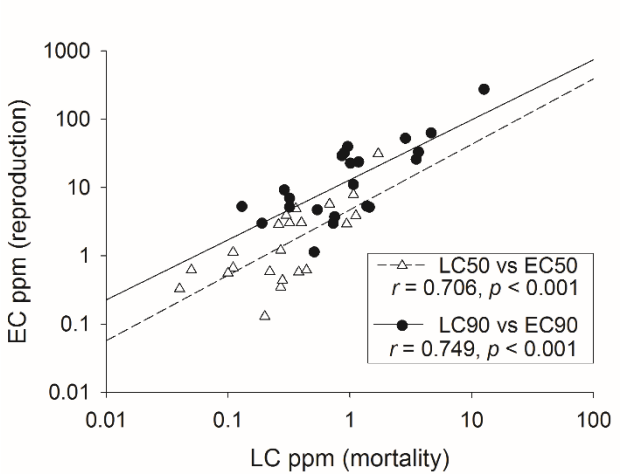

Figure 4.4. Correlation I of natural log-transformed LC and EC values (n = 22) for soybean aphid in leaf-dip bioassays with dashed line and solid lines representing least squares regression line for 50 and 90% values, respectively.

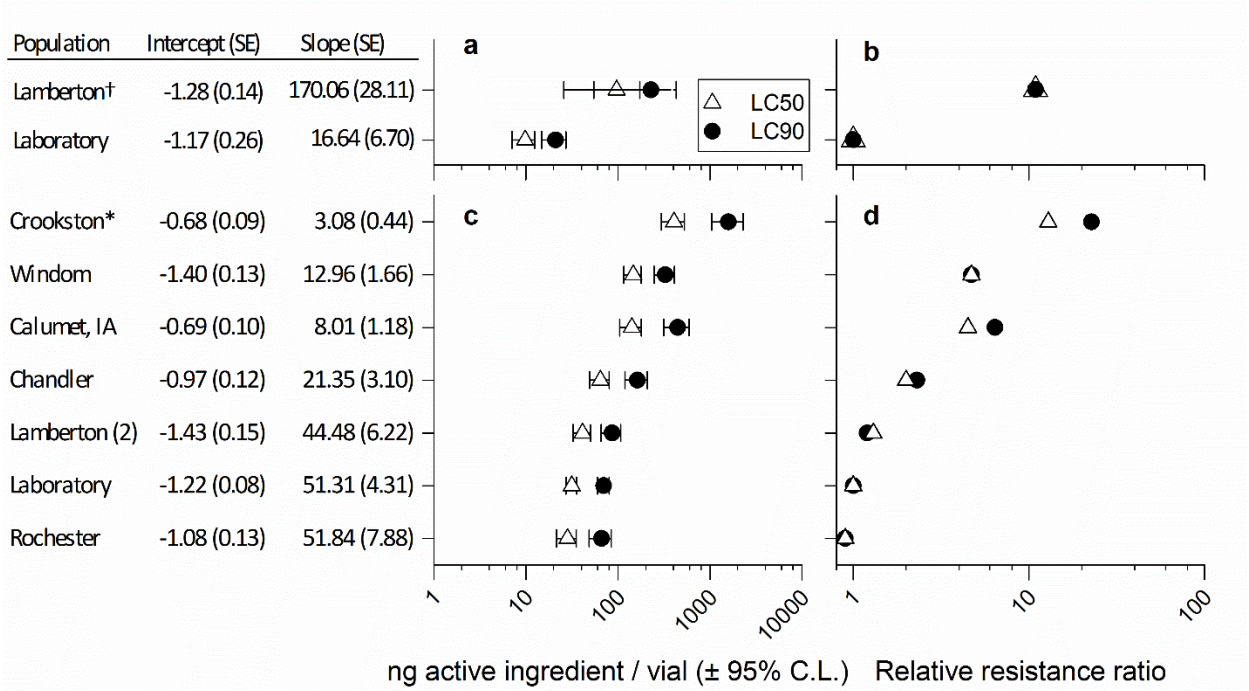

Figure 4.5. Amount of λ-cyhalothrin per vial causing 50 and 90% mortality (**a, c &e**) and relative resistance (**b, d & f**) of Minnesota soybean aphid populations in glass-vial bioassays from 2015 (**a** & **b**) and 2016 (**c** & **d**). Relative resistance ratio is the LC_{50} (ng/vial concentration at 50% mortality) or LC_{90} for a population divided by the corresponding LC of the laboratory population within a year. Concentrations were natural-log back-transformed. An asterisk (*) or dagger (†) indicates aphids collected after an application of λ-cyhalothrin or bifenthrin, respectively, failed to control aphid populations. 2015 Lamberton aphids were collected from the same field as the second collection of Lamberton aphids in the 2015 leaf-dip bioassay after a field application of bifenthrin failed to control aphid populations. Confidence intervals that do not overlap within LC_{50} or LC_{90}s indicate the estimates for the two populations are significantly different.

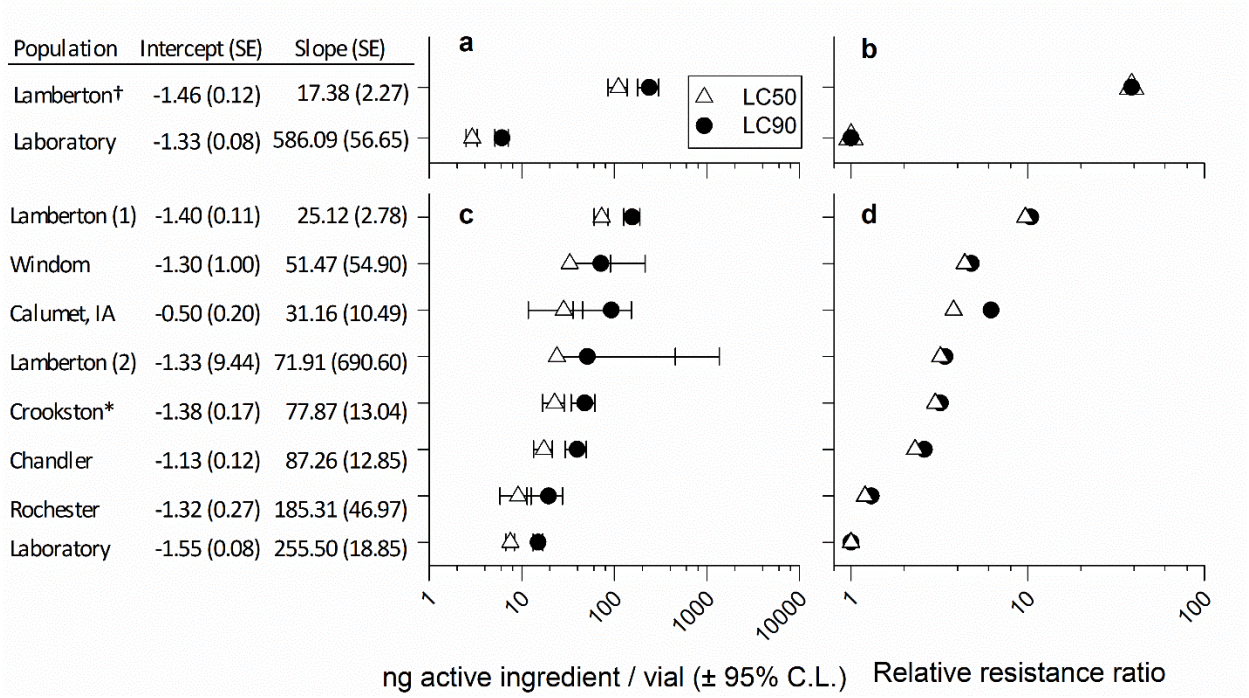

Figure 4.6. Amount of bifenthrin per vial causing 50 and 90% mortality (**a, c &e**) and relative resistance (**b, d & f**) of Minnesota soybean aphid populations in glass-vial bioassays from 2015 (**a** & **b**) and 2016 (**c** & **d**). Relative resistance ratio is the LC_{50} (ng/vial concentration at 50% mortality) or LC_{90} for a population divided by the corresponding LC of the laboratory population within a year. Concentrations were natural-log back-transformed. An asterisk (*) or dagger (†) indicates aphids collected after an application of λ-cyhalothrin or bifenthrin, respectively, failed to control aphid populations. 2015 Lamberton aphids were collected from the same field as the second collection of Lamberton aphids in the 2015 leaf-dip bioassay after a field application of bifenthrin failed to control aphid populations. Lower confidence intervals for Lamberton (2) and Windom are not included as they are truncated at zero. Confidence intervals that do not overlap within LC_{50} or LC_{90}s indicate the estimates for the two populations are significantly different.

Chapter 5: Interactions of host-plant resistance and foliar insecticides for soybean aphid management

5.1 Introduction

Soybean aphid (*Aphis glycines* Matsumura), a native to Asia, was first discovered in North America in Wisconsin in 2000 and has since spread through much of the soybean production area of the U.S. and Canada (reviewed by Ragsdale et al. 2004; Ragsdale et al. 2011). Soybean aphids feeding on phloem sap can reduce soybean seed size and number (Beckendorf et al. 2008), resulting in $2.4 billion in lost yield and control costs annually (Song et al. 2006; Kim et al. 2008). Soybean aphid has become the primary insect pest actively managed by soybean growers in North America (Hurley and Mitchell 2017).

Multiple management tactics have been explored as part of an integrated pest management (IPM) program to reduce soybean aphid population densities, but foliar insecticides, primarily pyrethroids and organophosphates, remain the primary tactic for outbreak suppression (reviewed by Hodgson et al. 2012). Foliar insecticide treatment is recommended when aphid populations reach 250 aphids per plant (i.e., economic threshold) to prevent economic injury which occurs when populations reach 674 aphids per plant (i.e., economic injury level) (Ragsdale et al. 2007; Koch et al. 2016). Prophylactic neonicotinoid seed treatments are also available, but they are unlikely to provide sufficient control for soybean aphid because concentrations in the plant generally decrease to negligible levels before aphid populations begin to build (Krupke et al. 2017).

Others factors that can limit soybean aphid population growth include predatory insects, parasitoids, and pathogens. Natural enemies of the soybean aphid include predatory Coccinellidae and Anthocoridae, and parasitic Hymenoptera (Ragsdale et al. 2011). The presence of some predators, such as coccinellids, can prevent or suppress aphid outbreaks, but natural enemies do not consistently keep aphids from reaching levels high enough to cause economic damage (Koch and Costamanga 2017). However, natural enemies are less common where more insecticides are used (reviewed by Weinzierl 2009; Hodgson et al. 2012).

Host-plant resistance is a cornerstone of many IPM programs, due to typically high levels of compatibility with other management tactics (Pedigo 1995). However, the initial incorporation of host-plant resistance into IPM is often slow (Stout and Davis 2009). Host-plant resistance is the use of pest-resistant plants to maintain pest populations at low levels or tolerate levels normally damaging to susceptible lines (reviewed by Painter 1958; Smith 2005). Aphid-resistant soybean lines containing *Rag* (i.e., Resistance to *Aphis glycines*) genes have been found that reduce aphid populations (e.g., Hill et al. 2004; Hesler and Dashiell 2007; Bansal et al. 2013; Bhusal 2013). These aphid-resistant plants can reduce population growth of or repel soybean aphids (Diaz-Montano et al 2006). However, these plants are not immune to soybean aphid, and eventually aphids can reach population sizes large enough to cause damage to the plant and affect yield (Hill et al. 2012; Hesler 2013). Particularly, some soybean aphid biotypes are able to overcome these resistance traits (Alt and Ryan-Mahmutagic 2013; Hesler et al. 2013), but the distribution of these biotypes is variable across the Upper Midwest (Cooper et al. 2015; Crossley and Hogg 2015).

Integration of host-plant resistance and insecticides can be useful in part because insects that feed on resistant plants can become more susceptible to insecticide than those that feed on susceptible plants (e.g., Heinrichs et al. 1984; Tabadkani et al. 2017). This effect has been demonstrated in soybean pests on resistant plants (Kea et al. 1978), but not for soybean aphid with foliar insecticides. Effects of combined management tactics can occur in three ways (reviewed by Eigenbrode and Trumble 1994; Quisenberry and Schotzko 1994). An independent effect occurs when two tactics that control a pest do not affect the efficacy of the other (i.e., an additive effect). A synergistic interaction provides more control than expected (i.e., enhanced pesticide efficacy) on treated resistant plants than treated susceptible plants. Conversely, an antagonistic interaction can reduce the effectiveness of another control tactic to the point that the combined tactics are no more effective than or even worse in efficacy than a single tactic. Both additive and synergistic effects can be beneficial to growers by reducing pest populations more than single tactics alone. Meanwhile, antagonistic effects that reduce efficacy could lead to a population being exposed to an insecticide without any economic benefit.

If the use of aphid-resistant plants can also allow for fewer insecticide applications, operating costs may decrease, and yields may increase in high-pressure locations due to decreases in plant stress. While host-plant resistance is often compatible with natural enemies, many insecticides are not (Desneux et al. 2007; Weinzierl 2009; Pezzini et al. 2015). Maintaining beneficial insect populations after treatment may also suppress soybean aphid population growth and prevent outbreaks of secondary pests. To test whether interactions may occur between aphid-resistant soybean plants and foliar insecticides, we examined the effects of these tactics alone and in combination on

soybean aphid and its predator populations under Minnesota field conditions over three years and in controlled greenhouse experiments.

7.2 Materials and Methods

Two near isogenic lines were used in both field and greenhouse experiments to represent the soybean aphid susceptible IA3027 (i.e., no known *Rag* genes) and the resistant IA3027RA1 (i.e., *Rag1* gene) genotypes (Wiarda et al. 2012; McCarville et al. 2014a). Three insecticides were used in field experiments, and two in greenhouse bioassays. The first was a formulated mixture of pyrethrins and azadirachtin (Azera®, MGK, Minneapolis, MN) available for use by organic and conventional growers. The remaining two conventional insecticides were an organophosphate, chlorpyrifos (Lorsban®, Dow Agrosciences, Inc., Indianapolis, IN), and a pyrethroid, λ-cyhalothrin (Warrior II with Zeon Technology®, Syngenta Crop Protection, Inc., Basel, Switzerland).

5.2.1 Field experiment

The field experiment was conducted in 2013, 2014, and 2015 near Rosemount, MN to measure effects of combinations of soybean varieties and insecticides on soybean aphids and associated predators. The design consisted of 32 plots arranged in a randomized complete block design with four replications (blocks) of eight treatments. Treatments were a fully-crossed 2×4 factorial treatment structure with two kinds of plant resistance (i.e., IA3027 or IA3027RA1) and four kinds of insecticide treatment (i.e., untreated, chlorpyrifos, λ-cyhalothrin, and the mixture of pyrethrin and azadirachtin) (eight treatments total per replications). However, in 2013, the mixture of pyrethrin and azadirachtin was not used as a treatment, so two more untreated plots were included for

both IA3027 and IA3027RA1 in addition to chlorpyrifos and λ-cyhalothrin treatments. Plots were four rows wide by 4.6 m long, planted at 2.5 cm depth, with 76-cm row spacing and 1.5 m alleys between plots. Plots were planted on 11 June 2013, 13 June 2014, and 27 May 2015 at a rate of approximately 39 seeds per m.

Beginning when soybeans emerged, plots were sampled weekly by whole-plant counts for soybean aphids and the two primary taxa of soybean aphid predators, Coccinellidae and the anthocorid, *O. insidiosus* on randomly selected plants. The number of plants inspected per plot depended upon aphid abundance. Twenty plants were inspected until 80% of plants were infested; then, sample size was reduced to 10 plants for the remainder of the season. In 2013, insecticides were applied on 15 July. In 2014 and 2015, insecticides were applied on 8 August and 11 August, respectively, after susceptible plots reached the economic threshold of 250 aphids per plant. Applications were made with a CO_2-pressurized backpack sprayer using a 3.05-m boom with eight nozzles (XR-Teejet 8002 flat fan, with no screen) and calibrated to deliver 187.04 liters/ha at 275.8 kPa. Chlorpyrifos, λ-cyhalothrin, and the mixture of pyrethrins and azadirachtin were applied at maximum labeled rates (i.e., 2.3, 0.12, and 4.1 L of product per ha, respectively). Aphid and predator sampling continued at 3, 7, and 14 d after treatment.

5.2.2 Greenhouse bioassays

The greenhouse bioassay was designed to measure aphid susceptibility as the main response variable. For λ-cyhalothrin and the mixture of pyrethrins and azadirachtin, separate leaf-dip insecticide bioassays were performed with a fully-crossed factorial

treatment structure with two kinds of plant resistance (i.e., IA3027 or IA3027RA1) and two kinds of insecticide treatment (i.e., treated or untreated). For each insecticide, the experiment was repeated over three dates with four replications of each treatment per date.

Aphids were sourced from a laboratory colony of biotype 1 soybean aphid (i.e., susceptible to aphid-resistant plants expressing *Rag* genes) that has not been exposed to insecticides since discovery in North America (Kim et al. 2008b). These aphids were reared at 25°C with a 14:10 (L:D) h photoperiod at 70% RH on aphid-susceptible Williams 82 soybean. Aphids were transferred to and reared on caged IA3027 and IA3027RA1 soybean in a greenhouse at 25°C with a 16:8 (L:D) h photoperiod for three days before bioassays to account for potential handling stress and acclimate aphids to these varieties.

Preliminary laboratory assays were performed to determine concentrations of λ-cyhalothrin and the mixture of pyrethrins and azadirachtin that would cause approximately 35 to 50% aphid mortality (data not shown). Concentrations of active ingredient(s) in 200 mL deionized water were 0.463 μL of λ-cyhalothrin or 1.6 mL of the mixture of pyrethrins and azadirachtin. IA3027 and IA3027RA1 plants were grown to V1 stage (Fehr and Caviness 1977) under greenhouse conditions (at 25°C with a 16:8 (L:D) h photoperiod) in (10 × 10 × 10 cm) pots filled with approximately 700 cm^3 of potting soil (Sunshine MVP, Sun Gro Horticulture Products). Seeds were planted at a depth of 2 cm and a 1-cm layer of sand was added to the top of the soil to minimize fungus gnat infestation (Harris et al. 1996). At time of treatment, a unifoliate leaf on each plant was dipped and gently agitated for 10 seconds in water (i.e., untreated control) or insecticide

solution and then allowed to dry to 60 minutes. Ten apterous adult aphids were then transferred to the abaxial side of the previously dipped leaf and were confined in a clip cage attached to the leaves (e.g., Davis et al. 2005; Hanson et al. 2016b). Mortality of the aphids was recorded after 48 hours, with mortality defined as lack of movement after being prodded with a fine-tipped paintbrush. Living aphids typically were feeding or actively walking around inside the cages.

5.2.3 Statistical analysis

Aphid counts and predator counts from the field experiment were used to calculate cumulative insect days (CAD for aphids and CPD for predators) to characterize for insect abundance over time

$$CAD\ or\ CPD = \sum_{i=1}^{n} \frac{x_i + x_{i-1}}{2} * t_i \text{ [eq.1]}$$

where *n* is the number of sampling dates, *x* is the average number of aphids, coccinellids, or *O. insidiosus* per plant in a plot on observation date *t*, and t_i is days since the previous sample date (Ruppel 1983; Hanafi et al., 1989). CAD and CPD were calculated from soybean emergence to 14 d after insecticide application. The change in CAD from the last pre-treatment sample date to 14 d after insecticide application was also calculated to more specifically assess the effects and interactions of the insecticides.

Data were analyzed by two-way ANOVA with main effects of plant genotype (i.e., susceptible and *Rag1*) and insecticide treatment (i.e., control, chlorpyrifos, λ-cyhalothrin, and the mixture of pyrethrins and azadirachtin) and the two-way interaction. Independent (i.e., additive) effects of genotype and insecticide (i.e., identical slopes for treated and

untreated groups) occurred when mean CAD for both untreated resistant plants and insecticide-treated susceptible plants were significantly different from untreated susceptible plants without an interaction effect. Significant interaction effects (i.e., nonparallel slopes) indicated either synergistic effects of resistant plants and insecticide that increased control, or antagonistic effects that decrease control. CAD and predator-prey ratios (i.e., CPD per CAD for each predator taxa to account for aphid abundance affecting predator abundance) were analyzed using PROC GLM from SAS 9.4 with log-transformed response variables to fulfill assumptions of homogeneity of variance and normality. Mortality data from greenhouse bioassays were also analyzed by ANOVA with PROC GLIMMIX using a binomial distribution with effects for genotype, insecticide and the interaction.

7.2 Results

In the field study, mean season-long CAD in untreated susceptible plots ranged from 325 in 2013 to 22,473 in 2014 (Fig 5.1). Season-long CAD in resistant soybean compared to susceptible soybean in each year (Table 5.1; Fig. 5.1 a-c). Across soybean genotypes, plots treated with chlorpyrifos or λ-cyhalothrin had significantly lower season-long CAD compared to untreated plots in each year, except for chlorpyrifos in 2015. Plots treated with the mixture of pyrethrins and azadirachtin had season-long CAD similar to the untreated plots (Table 5.1; Fig. 5.1 b-c). Significant synergistic interactions (i.e., pest suppression greater than additive effects of each tactic) were found between the resistant genotype and λ-cyhalothrin in 2013 and chlorpyrifos in 2014 (Table 5.1). Resistant plots treated with λ-cyhalothrin or chlorpyrifos (except for chlorpyrifos in 2015) had significantly fewer CAD than all other treatments (Fig. 5.1c). From 2013 to

2015, season-long predator-prey ratios did not significantly differ among any treatments for Coccinellidae [$F(7,24) < 0.85$, $p > 0.53$] or *O. insidiosus* [$F(7, 24) < 1.82$, $p > 0.13$].

Increases in CAD after insecticide treatment were significantly lower in resistant plots compared to susceptible plots across years (Table 5.1; Fig. 5.1 d-f). The mixture of pyrethrins and azadirachtin did not decrease CAD growth for either genotype (Fig 5.1; Table 5.1). Synergistic interactions occurred between the resistant genotype and λ-cyhalothrin in 2013 and 2015 (Fig. 5.1 a & c; Table 5.1). However, an antagonistic interaction occurred between the resistant genotype and chlorpyrifos in 2015 (Table 5.1; Fig. 5.1c). The increase in CAD after treatment with chlorpyrifos was greater on the resistant genotype compared to the susceptible genotype (Fig. 5.1 c). The increase in CAD after chlorpyrifos treatment in 2013 to 2015 and λ-cyhalothrin treatment in 2014 was significantly lower than untreated plots for susceptible plants. Chlorpyrifos or λ-cyhalothrin-treated resistant plots had less CAD growth than untreated resistant plots in 2013 and 2014, but this only occurred for λ-cyhalothrin plots in 2015.

In the greenhouse bioassays, aphid mortality across genotypes ranged from 7% to 93%% for λ-cyhalothrin and 6% to 60% for the mixture of pyrethrins and azadirachtin. Mortality differed significantly by genotype and insecticide treatment (Table 5.3; Fig. 5.2). However, no significant interactions were found between soybean genotype and either insecticide, which indicated effects of these tactics were additive (Table 5.3).

5.4 Discussion

Under field conditions, we demonstrated that both synergistic and antagonistic interactions can occur between effects of resistant varieties and foliar insecticides on soybean aphid abundance. Overall, season-long CAD for chlorpyrifos and λ-cyhalothrin showed either independent or synergistic relationships between host-plant resistance and foliar insecticides, but these effects were not consistent across years. While chlorpyrifos in 2015 did not have a significant interaction effect, chlorpyrifos-treated resistant plants did not have fewer CAD than untreated resistant plants (Fig 5.1c). Conversely, the change in CAD in the 14 d after insecticide application indicates a significant antagonistic interaction occurred for chlorpyrifos in 2015 (Table 5.1). Likewise, there was not a significant λ-cyhalothrin interaction in 2015 for season-long CAD, but there was a synergistic interaction while examining the change in CAD post-treatment. These differences in CAD measures seem to suggest that while interactions can occur, they may not always be strong enough to affect season-long CAD, which is the metric used for assessing the economic injury level (Ragsdale et al. 2007).

Under greenhouse conditions, no interactions were found in the bioassays with λ-cyhalothrin or the mixture of pyrethrins and azadirachtin. These experiments would have eliminated potential confounding factors that occur under field conditions such as aphid biotype, weather conditions on insecticide efficacy, or natural enemies. Regardless of plant genotype, the mixture of pyrethrins and azadirachtin did not have fewer CAD in the field than untreated plants, but mortality was higher than untreated plants in the greenhouse bioassay. Pyrethrins break down quickly when exposed to sunlight, so it is possible environmental conditions explain the differences in efficacy between field and

greenhouse settings (Singh et al. 2010). Λ-cyhalothrin exhibited a synergistic interaction in the 2013 field study, so the interaction variability across years could be due to factors not present in greenhouse conditions.

Other studies have looked for interaction effects of host-plant resistance and insecticide seed treatments for soybean aphid. McCarville and O'Neal (2013) examined potential interactions of thiamethoxam seed treatments and aphid-resistant soybean. The only interaction that occurred was due to populations being so small on a *Rag1* and *Rag2* pyramided line that aphid populations could not be reduced further by insecticide treatment (McCarville and O'Neal 2013). Kandel et al. (2015) did not find an interaction between thiamethoxam and aphid-resistant soybean in a one year field study.

Previous studies have shown that coccinellid populations are lower on *Rag1* aphid-resistant plants potentially through direct effects of the plants on predators or decreased aphid availability (Lundgren et al. 2009; Kandel et al. 2015). After accounting for aphid abundance in this study by analyzing predator prey ratios, we did not find any differences for Coccinellidae or *O. insidiosus* in any treatment. Using reduced-risk pesticides can reduce non-target effects on predators (Kraiss & Cullen 2008; Pezzini et al. 2015), so an insecticide that produces synergistic effects for soybean aphid could have different effects on natural enemies than those used in our experiments.

Various mechanisms for synergistic and antagonistic interactions between management tactics have been proposed. Synergistic effects are primarily thought to be caused by reduce body weight and availability of physiological resources to detoxify insecticides due to reduced feeding (Eigenbrode and Trumble 1994; Quisenberry and

Schotzko 1994). Antagonistic effects can occur in part because of an insect's detoxification mechanisms becoming upregulated from exposures to stressors (Eigenbrode and Trumble 1994; Quisenberry and Schotzko 1994). Phytochemicals involved in plant defense can lead to increased tolerance to pesticides in another soybean pest, the two-spotted spider mite, *Tetranychus urticae* Koch (Dermauw et al. 2013). Gossypol found in pest-resistant cotton can increase enzyme activity related to insecticide detoxification in the cotton leafworm, *Alabama argillacea* Hubner (El-Sebae et al. 1981). Pesticide resistance in pests can also enhance the ability to tolerate phytochemicals (Bagchi et al. 2016). Cytochrome P450s are often discussed as a mechanism to overcome both host-plant resistance and insecticide susceptibility (Scott et al. 1998), which raises the possibility of cross resistance when exposure to one or both control tactics (Quisenberry and Schotzko 1994). While reduced feeding could weaken defense mechanisms of an insect, reduced feeding can also limit oral exposure to insecticides (Abro and Wright 1989). Plant structures contributing to pest resistance, such as trichomes, can also affect insecticide coverage on the plant, and changes in within-plant distribution of the pest may also affect insecticide exposure (Quisenberry and Schotzko 1994).

Soybean aphid biotypes, which vary in reaction to *Rag* genes, may also play a role in when interaction effects occur. As *Rag1* plants in our study were effective at reducing aphid abundance, field populations likely consisted primarily of biotype 1 aphids. However, other biotypes could also have been present on the same plants. Studies have not yet been performed to see if baseline susceptibility to insecticides varies across soybean aphid biotypes, but increased resistance to λ-cyhalothrin had been confirmed in

other parts of the state each year this study was performed (Chapter III; Hanson et al. 2016b). Variation in biotype resistance to insecticides could have played a role in differences in insecticide efficacy. Furthermore, the mechanisms for soybean aphid resistance to insecticides and virulence to host-plant resistance generally remain unknown. Soybean aphid biotypes have baseline fitness differences when reared on susceptible plants (Varenhorst et al. 2015a). Potential biotype effects are further complicated as induced plant susceptibility can occur where normally avirulent aphids can successfully feed on resistant plants if virulent aphids had been previously feeding on the plant (Varenhorst et al. 2015b). Only biotype 1 was used in the greenhouse bioassays where no interactions were found, so factors associated with biotypes may be one possible explanation for only finding interaction effects in the field.

This study demonstrates over three years in the field and in a greenhouse study that variable interactions between foliar insecticide and aphid-resistant soybean can occur. In most cases, host-plant resistance and foliar insecticide use had independent (i.e., additive) or synergistic effects on aphid control. One instance of antagonism was found, but the insecticide-treated aphid-resistant plants did not have higher CAD than untreated aphid-resistant plants. These results and the lack of effects on predator-prey ratios suggest that host-plant resistance and foliar insecticide use are compatible for soybean aphid management.

Tables 5.5

Table 5.1. Linear regression effects of soybean genotype and foliar insecticide treatment on season-wide CAD (cumulative aphid days) and change in CAD after insecticide treatment.

			Season-long CAD			Post-treatment CAD[2]		
Year	Effect	DF	Est. (SE)[1]	*F*	*p*	Est. (SE)[1]	*F*	*p*
2013	Genotype	1,26		113.19	<0.01		115.61	<0.01
	Insecticide:	2,26						
	chlorpyrifos			19.68	<0.01		50.10	<0.01
	λ-cyhalothrin			15.09	<0.01		25.19	<0.01
	Genotype x Insecticide:	2,26						
	chlorpyrifos		-0.48 (0.36)	1.71	0.20	-0.68 (0.33)	4.24	0.05
	λ-cyhalothrin		-1.02 (0.36)	7.84	<0.01	-0.98 (0.33)	8.82	<0.01
2014	Genotype	1,24		112.56	<0.01		72.39	<0.01
	Insecticide:	3,24						
	pyrethrins + azadirachtin			0.25	0.61		1.17	0.29
	chlorpyrifos			20.43	<0.01		64.06	<0.01
	λ-cyhalothrin			30.68	<0.01		98.04	<0.01
	Genotype x Insecticide:	3,24						
	pyrethrins + azadirachtin		-0.28 (0.58)	0.23	0.61	-0.31 (0.49)	0.41	0.53
	chlorpyrifos		-1.38 (0.58)	5.66	0.03	-0.87 (0.49)	3.13	0.09
	λ-cyhalothrin		-0.86 (0.58)	2.22	0.15	-0.04 (0.49)	0.01	0.94
2015	Genotype	1,24		70.58	<0.01		22.34	<0.01
	Insecticide	3,24						
	pyrethrins + azadirachtin			0.74	0.40		0	0.95
	chlorpyrifos			2.18	0.15		68.87	<0.01
	λ-cyhalothrin			12.78	<0.01		84.06	<0.01
	Genotype x Insecticide:	3,24						
	pyrethrins + azadirachtin		0.04 (0.32)	0.02	0.90	0.46 (0.63)	0.55	0.48
	chlorpyrifos		0.37 (0.32)	1.35	0.26	4.78 (0.63)	58.22	<0.01
	λ-cyhalothrin		-0.48 (0.32)	2.31	0.14	-4.61 (0.63)	54.32	<0.01

[1] Negative parameter estimates indicate synergistic interactions and positive parameter estimates indicate antagonistic interactions.

[2] Calculated as change in CAD between the latest pre-treatment sample date and 14 d after insecticide treatment.

Table 5.2. Logistic regression of soybean aphid mortality in greenhouse bioassay.

Insecticide	Effect	*DF*	*F*	*p*
λ-cyhalothrin	Genotype	1,39	99.11	< 0.001
	Insecticide	1,39	55.84	< 0.001
	Genotype x Insecticide	1,39	0.93	0.340
Pyrethrins + azadirachtin	Genotype	1,40	46.67	< 0.001
	Insecticide	1,40	26.58	< 0.001
	Genotype x Insecticide	1,40	3.48	0.070

5.6 Figures

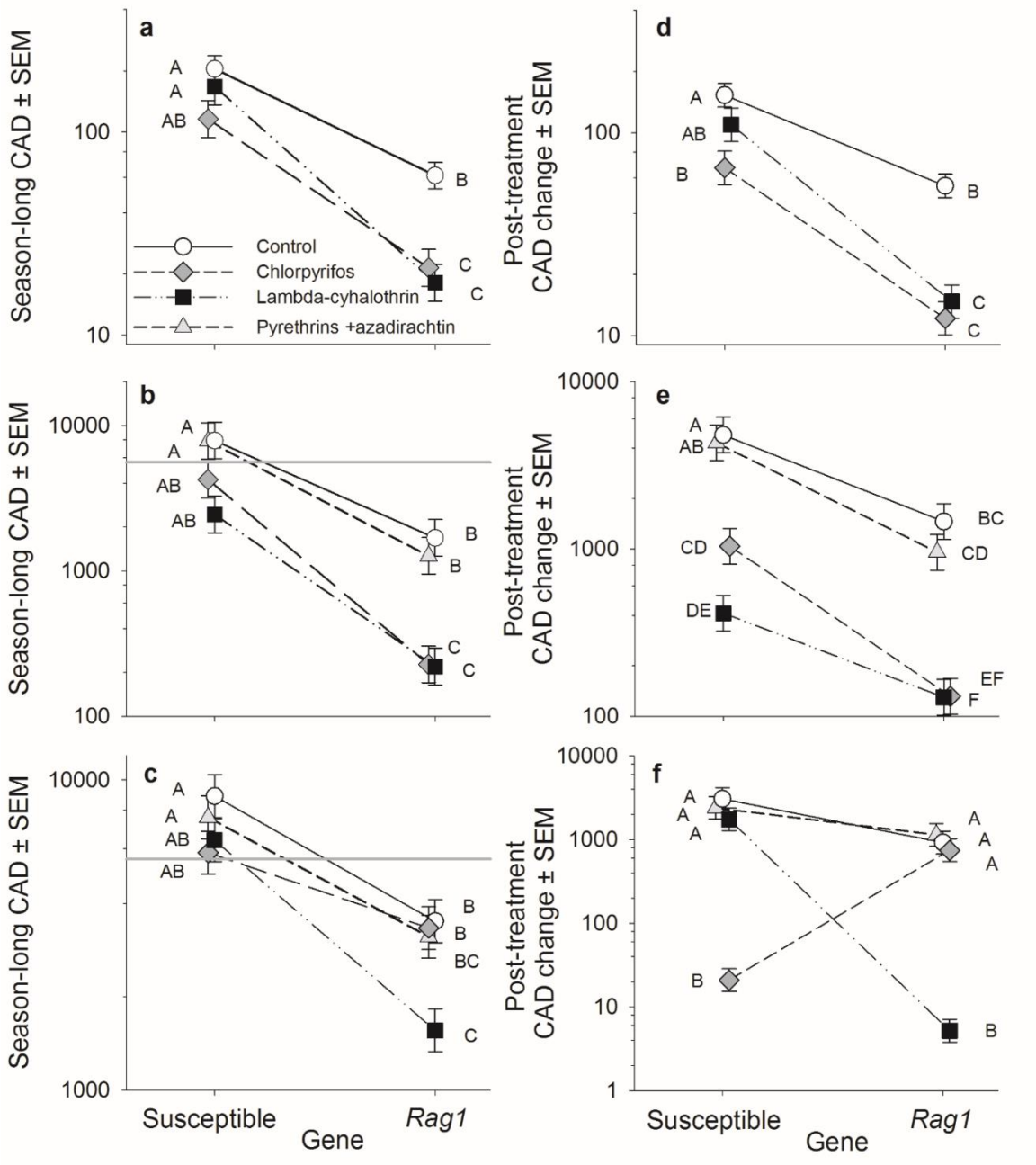

Figure 5.1. Mean season-wide CAD (cumulative aphid days) for soybean aphid (**a**, **b** & **c**) and mean change in CAD after insecticide treatment (**d**, **e** & **f**) in 2013 **(a & d)**, 2014 **(b & e)**, and 2015 **(c & f)**. Different letters adjacent to means indicate significant differences according to the Tukey-Kramer test for multiple comparisons at ($\alpha = 0.05$). The gray horizontal line in **b** and **c** represents the economic injury level of 5563 CAD.

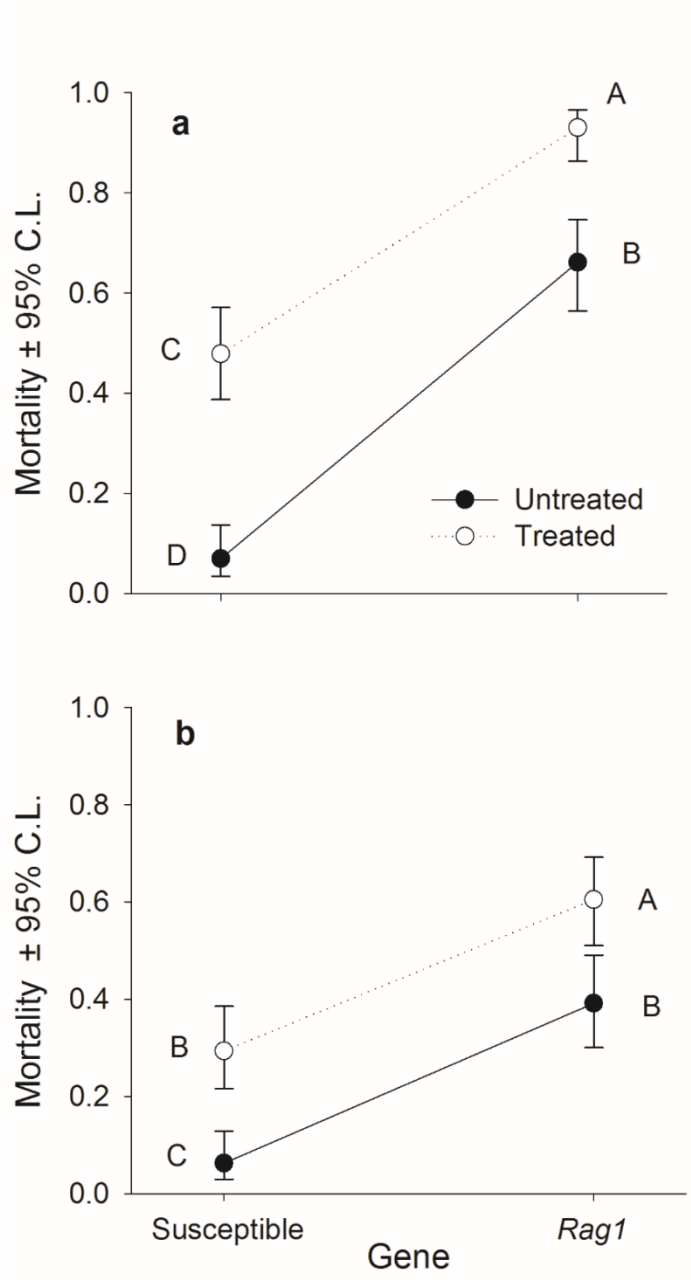

Figure 5.2. Mean propotion mortality of aphids on susceptible and aphid-resistant plants in greenhouse bioassays with **(a)** λ-cyhalothrin and **(b)** a mixture of pyrethrins and. Different letters adjacent to means indicate significant differences according to the Tukey-Kramer test for multiple comparisons at ($\alpha = 0.05$). Insecticide concentrations below the label rate were used in order to achieve approximately 35-50% mortality of soybean aphid on insecticide-treated susceptible plants.

Bibliography

Abro, G. H. and D. J. Wright. 1989. Host plant preference and influence of different cabbage cultivars on the toxicity of abamcctin and cypermethrin against *Plutella xylostella*. Ann. Appl. Bio. 115: 461-467.

Ahmad, M., M. I. Arif, and I. Denholm. 2003. High resistance of field populations of the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) to pyrethroid insecticides in Pakistan. J. Econ. Entomol. 96: 875-878.

Ahmad, M. and S. Akhtar. 2013. Development of insecticide resistance in field populations of *Brevicoryne brassicae* (Hemiptera: Aphididae) in Pakistan. J. Econ. Entomol. 106: 954-958.

Ajayi-Oyetunde, O. O., B. W. Diers, D. Lagos-Kutz, C. B. Hill, G. L. Hartman, U. Reuter-Carlson, C. A. Bradley. 2016. Differential reactions of soybean iolines with combinations of aphid resistance genes *Rag1*, *Rag2*, and *Rag3* to four doybean aphid biotypes. J. Econ. Entomol .2016; 109: 1431-1437.

Alt, J., and M. Ryan-Mahmutagic. 2013. Soybean aphid biotype 4 identified. Crop Sci. 53:1491–1495. Doi:10.2135/cropsci2012.11.0672

Agrawal, A. A., S. Tuzun and E. Bent. 1999. Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture. APS Press, St. Paul.

Bagchi, V. A., J. P. Siegel, M. R. Demkovich, L. N. Zehr, and M. R. Berenbaum. 2016. Impact of pesticide resistance on toxicity and tolerance of hostplant phytochemicals in *Amyelois transitella* (Lepidoptera: Pyralidae). J Insect Sci. 17: 47.

Bahlai, C. A., A. W. Schaafsma, D. Lagos, D. Voegtlin, J. L. Smith, J. A. Welsman, Y. Xue, C. DiFonzo, and R. H. Hallett. 2014. Factors associated with winged forms of soybean aphid and an examination of North American spatial dynamics of this species in the context of migratory 117aculate. Agr. Forest Entomol. 16: 240–250.

Bales, C., G. Zhang, M. Liu, C. Mensah, C. Gu, Q. Song, D. Hyten, P. Cregan, D. Wang. 2013. Mapping soybean aphid resistance genes in PI 567598B. Theor. Appl. Genet. 126: 2081–2091

Bandillo, N., D. Jarquin, Q. Song, R. Nelson, P. Cregan, J. Specht, A. Lorenz. 2015. A population structure and genome-wide 117aculate117n117 analysis on the USDA soybean germplasm collection. Plant Genome. 8: 1-13.

Bansal R., M. A. R. Mian, and A. P. Michel. 2013. Identification of novel sources of host plant resistance to known soybean aphid biotypes. J. Econ. Entomol. 106:1479-1485.

Bao, Y., J. E. Kurle, G. Anderson, and N. D. Young. 2015. Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Mol. Breed. 35: 128.

Barrett, J. C., B. Fry, J. Maller, and M. J. Daly. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Beckendorf, E. A., M. A. Catangui, and W. E. Riedell. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron. J., 100: 237-246.

Bergfelder-Drüing, S., C. Grosse-Brinkhaus, B. Lind, M. Erbe, K. Schellander, H., Simianer, E. Tholen. 2015. A genome-wide association study in large white and landrace pig populations for number piglets born alive. PloS one 10: e0117468.

Bhusal, S. J., G. Jiang, K. J. Tilmon, and L. S. Hesler. 2013. Identification of soybean aphid resistance in early maturing genotypes of soybean. Crop Sci. 53:491-499.

Bhusal, S. J., G. Jiang, L. S. Hesler, and J. H. Orf. 2014. Soybean aphid resistance in soybean germplasm accession of maturity group I. Crop Sci. 54:2093–2098.

Bhusal, S. J., G. L. Jiang, Q. Song, P. B. Cregan, D. Wright, J. L. Gonzalez-Hernandez. 2017. Genome-wide detection of genetic loci associated with soybean aphid resistance in soybean germplasm PI 603712. *Euphytica*, 213: 144.

Bi, R., Y. Pan, Q. Shang, T. Peng, S. Yang, S. Wang, X. Xin, Y. Liu, and J. Xi. 2016. Comparative proteomic analysis in *Aphis glycines* Mutsumura under lambda-cyhalothrin insecticide stress. Comp. Biochem. Physiol. Part D Genomics Proteomics 19: 90-96.

Bielza, P. 2008. Insecticide resistance management strategies against the western flower thrips, *Frankliniella occidentalis*. Pest. Manag. Sci., 64: 1131–1138.

Brace, R.C. and Fehr, W.R. 2012. Impact of combining the *Rag1* and *Rag2* alleles for aphid resistance on agronomic and seed traits of soybean. Crop Sci. 52: 2070-2074.

Brunner, S. M., R. J. Goos, S. J. Swenson, S. P. Foster, B. G. Schatz, Y. E. Lawley, and D. A. Prischmann-Voldseth. 2014. Impact of nitrogen fixing and plant-growth promoting bacteria on a phloem feeding soybean herbivore. Appl. Soil. Ecol. 86:71-81.

Cao, H. H., M.Z. Pan, H. R. Liu, S. H. Wang, T. X. Liu. 2015. Antibiosis and tolerance but not antixenosis to the grain aphid, Sitobion avenae (Hemiptera: Aphididae), are essential mechanisms of resistance in a wheat cultivar. Bull. Entomol. Res. 105: 448-455.

Catangui, M. A., E. A. Beckendorf, and W. E. Riedell. 2009. Soybean aphid population dynamics, soybean yield loss, and development of stage-specific economic injury levels. Agro. J. 101: 1080–1092.

Chandrasena, D., C. DiFonzo, and A. Byrne. 2011. An aphid-dip bioassay to evaluate susceptibility of soybean aphid (Hemiptera: Aphididae) to pyrethroid, organophosphate, and neonicotinoid insecticides. J. Econ. Entomol. 104: 1357-1363.

Chandrasena, D., Y. Wang, C. Bales, J. Yuan, C. Gu, D. Wang. 2015. Pyramiding *rag*3, *rag*1b, *rag*4, and *rag*1c aphid resistant genes in soybean germplasm. Crop Sci. 55.

Chang, H., and G. L. Hartman. 2017. Characterization of insect resistance loci in the USDA soybean germplasm collection using genome-wide association studies. Front. Plant Sci. 8:670.

Chiozza, M. V., M. E. O'Neal, and G. C. MacIntosh. 2010. Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant

soybean plants in response to the soybean aphid (Hemiptera: Aphididae). Environ. Entomol. 39: 856-864.

Chirumamilla, A., C. B. Hill, and G .L. Hartman. 2014. Stability of soybean aphid resistance in soybean across different temperatures. Crop. Sci. 54:2557–2563.

Chung, G., R. J. Singh. 2008. Broadening the genetic base of soybean: a multidisciplinary approach. Cr. Rev. Plant Sci. 27: 295-341.

Collard, B. C. Y., M. Z. Z. Jahufer, J. B. Brouwer, E. C. K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica, 142: 169-196.

Cooper, S. G. , V. Concibido, R. Estes, D. Hunt, G. Jiang,C. Krupke, B. McCornack, R. Mian, M. O'Neal, V. Poysa, D. Prischmann-Voldseth, D. Ragsdale, N. Tinsley, and D. Wang. 2015. Geographic distribution of soybean aphid biotypes in the United States and Canada during 2008–2010. Crop Sci. 55: 2598-2608.

Cornelis, M. C., A. Agrawal, J. W. Cole, N. N. Hansel, K. C. Barnes, T. H. Beaty, S. N. Bennett, L. J. Bierut, E. Boerwinkle, K. F. Doheny, B. Feenstra, E. Feingold, M. Fornage, C. A. Haiman, E. L. Harris, M. G. Hayes, J. A. Heit, F. B. Hu, J. H. Kang, C. C. Laurie, H. Ling, T. A. Manolio, M. L. Marazita, R. A. Mathias, D. B. Mirel, J. Paschall, L. R. Pasquale, E. W. Pugh, J. P. Rice, J. Udren, R. M. van Dam, X. Wang, J. L. Wiggs, K. Williams, and K. Yu. 2010. The gene, environment association studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. Genet. Epidemiol. 34: 364–372.

Crossley, M.S. and D.B. Hogg. 2015. *Rag* virulence among soybean aphids (Hemiptera: Aphididae) in Wisconsin. J. Econ. Entomol. 108:326-338

Davis, J. A., E. B. Radcliffe, and D. W. Ragsdale. 2005. Soybean aphid, *Aphis glycines* Matsumura, a new vector of *potato virus Y* in potato. Am. J. Potato. Res. 82:197-201.

Dermauwa, W., N. Wybouwa, S. Rombautsb, B. Mentend, J. Vontase, Mi. Grbićf, R. M. Clark, R. Feyereisenj, and T. Van Leeuwena. 2013. A link between hostplant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proc. Natl. Acad. Sci. USA 110: E113–E122.

Desneux N, A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106

Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J. Econ. Entomol. 99:1884-1889.

Douglas, M. R. and J. F. Tooker. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. Environ. Sci. Technol., 49: 5088-5097.

Eigenbrode, S. D., J. T. Trumble. 1994. Host plant resistance to insects in integrated pest management in vegetable crops. J. Agric. Entomol, 11: 201-224.

El-Sabae, A. H., S. I. Sherby and N. A. Mansour. 1981. Gossypol as an inducer or inhibitor in *Spodoplera littoralis*. J Environ Sci Health B 16: 167·178.

Endelman, J.B. 2011. Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome 4:250-255.

Enders, L., R. Bickel, J. Brisson, T. Heng-Moss, B. Siegfried, A. Zera, and N. Miller. 2014. Soybean aphid (Hemiptera: Aphididae) response to soybean plant defense: stress levels, tradeoffs, and cross-virulence. Environ. Entomol. 43:47-57.

Faeth, S. H. and T. L. Bultman. 1986. Interacting effects of increased tannin levels on leaf-mining insects. Entomol. Exp. Appl. 40: 297-301

Faraggi, D., P. Izikson, and B. Reiser, 2003. Confidence intervals for the 50 per cent response dose. Stat. Med. 22: 1977–1988.

Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. Iowa State University Cooperative Extension Service Special Rep. 80. Iowa State University, Ames, IA.

Feng, R., J. G. Houseman, A. E. R. Downe, J. Atkinson, and J. T. Arnason. 1992. Effects of 2, 4- Dihydroxy-7- methoxy-1, 4-benzoxazin-3-one (DIMBOA) and 6-methoxybenzoxazolinone (MBOA) on the detoxification processes in the larval midgut of the European corn borer. Pest Biochem. Physiol. 44:147–154.

Feuillet, C., P. Langridge, and R. Waugh. 2008. Cereal breeding takes a walk on the wild side. Trends Genet. 24: 24-32.

Ffrench-Constant, R. H. and C. Bass. 2017. Does resistance really carry a fitness cost? Curr. Opin. Insect Sci. 21: 39-46.

Foster, S. P., G. Devine, and A. L. Devonshire. 2007. Insecticide resistance in aphids. P. 261-285. In H. van Emden and R. Harrington, eds. Aphids as Crop Pests. CAB Int., Oxfordshire, UK.

Frewin, A. J., A. W. Schaafsma, and R. H. Hallett. 2012. Susceptibility of *Aphelinus certus* to foliar-applied insecticides currently or potentially registered for soybean aphid control. P. M. Sci. 68: 202-208.

Gray, M. E. S. T. Ratcliffe, and M. E. Rice. 2009. The IPM paradigm: concepts, strategies, and tactics. In: Radcliffe, E. B., Hutchison, W. D., & Cancelado, R. E. (Eds.). *Integrated pest management: concepts, tactics, strategies and case studies*. Cambridge University Press.

Guillebeau, L. P., J. N. All, and A. M. Javid. 1989. Influence of weather on efficacy of pyrethroid insecticides for boll weevil (Coleoptera: Curculionidae) and bollworm (Lepidoptera: Noctuidae) in cotton. J. Econ. Entomol: 82: 291-296.

Hanafi, A., E. B. Radcliffe, and D. W. Ragsdale. 1989. Spread and control of potato leafroll virus in Minnesota. J. Econ. Entomol. 82:1201–1206. Doi:10.1093/jee/82.4.1201

Hanson, A. A., R. C. Venette, and J. P. Lelito. 2013. Cold tolerance of Chinese emerald ash borer parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) Biol. Control 67: 516–529.

Hanson, A., S. J. Bhusal, A. Lorenz, R. Koch. 2016a. Aphid-resistant soybean varieties for Minnesota. University of Minnesota Extension. www.extension.umn.edu/agriculture/soybean/pest/soybean-aphid/aphid-resistant-soybean

Hanson, A. A., J. H. Orf, and R. L. Koch. 2016b. Sources of soybean aphid resistance in early-maturing soybean germplasm. Crop Sci. 56: 154-163.

Hardman, J. M., D. L. Moreau, M. Snyder, S. O. Gaul, and E. D. Bent. 2000. Performance of a pyrethroid-resistance strain of the predator mite *Typhlodromus*

pyri (Acari: Phytoseiidae) under different insecticide regimes. J. Econ. Entomol. 93: 590–604.

Harris M.A., W.A. Gardner, and R.D. Oetting. 1996. A review of the scientific literature on fungus gnats (Diptera: Sciaridae) in the genus *Bradysia*. J. Entomol. Sci. 31: 252-276.

Hazard. B., X. Zhang, M. Naemeh, and J. Dubcovsky. 2014. Registration of durum wheat germplasm lines with combined mutations in *SBElla* and *SBEllb* genes conferring increased amylose and resistant starch. J. Plant. Regist. 8:334–338.

Heidel-Baker, T. 2012. Compatibility of soybean aphid integrated pest management strategies. Ph.D. diss., Univ. of Minnesota., St. Paul.

Heimpel, G.E., L. E. Frelich, D. A. Landis, K. R. Hopper, K. A. Hoelmer, Z. Sezen, M. K. Asplen, K. Wu. 2010. European buckthorn and Asian soybean aphid as components of an extensive invasional meltdown in North America. *Biol. Inv.* 12: 2913-2931.

Heinrichs, E. A., L. T. Fabellar, R. P. Basilio, T. C. Wen, F. Medrano. 1984 Susceptibility of rice plant hoppers, *Nilaparvata lugens* and *Sogatella furcifera* to insecticides as influenced by level of resistance in the host plant. Environ. Entomol. 13: 455–458.

Heng-Moss, T. M., X. Ni, T. Macedo, J. P. Markwell, F. P. Baxendale, S. S. Quisenberry, V. Tolmay. 2003. Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae) – infested wheat isolines. J. Econ. Entomol. 96:475–481.

Hernandez-Garcia, C. M. 2013. Seedcoat Pigmentation in Soybean: An RNA Silencing Mediated Trait. http://articles.extension.org/pages/61175/seedcoat-pigmentation-in-soybean:-an-rna-silencing-mediated-trait

Hesler, L. S. 2013. Resistance to soybean aphid among wild soybean lines under controlled conditions. Crop Prot. 53: 139-146. Doi:10.1016/j.cropro.2013.06.016.

Hesler, L. S., and K. E. Dashiell. 2007. Resistance to *Aphis glycines* (Hemiptera: Aphididae) in various soybean lines under controlled laboratory conditions. J. Econ. Entomol. 100:1464-1469.

Hesler, L. S. and K. E. Dashiell. 2008. Identification and characterization of new sources of resistance to *Aphid glycines* Matsumura (Hemiptera: Aphididae) in soybean lines. Appl. Entomol. Zool. 43:197-206.

Hesler, L. S., and K. E. Dashiell. 2011. Antixenosis to the soybean aphid in soybean lines. Open Entomol. J. 5:39-44.

Hesler, L. S., K. E. Dashiell, and J. G. Lundgren. 2007. Characterization of resistance to *Aphis glycines* in soybean accessions. Euphytica 154:91-99.

Hesler, L. S., D. A. Prischmann, and K E. Dashiell. 2012. Field and laboratory evaluations of soybean lines against soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 105:608-615.

Hesler, L. S., M. V. Chiozza, M. E. O'Neal, G. C. MacIntosh, K. J. Tilmon, D. I. Chandrasena, N. A. Tinsely, S. R. Cianzio, A. C. Costamagna, E. M. Cullen, C. D. DiFonzo, B. D. Potter, D. W. Ragsdale, K. Steffey, and K. J. Koehler. 2013. Performance and prospects of *Rag* genes for management of soybean aphid. Entomol. Exp. Appl. 147:201–216. Doi:10.1111/eea.12073

Hesler L., E. Beckendorf, K. Tilmon, N.R.B. Schultz, B.M. Va De Stroet, P. Rozenboom. 2017. https://npgsweb.ars-grin.gov/gringlobal/method.aspx?id=495901)

Hill, C.B., Y. Li, and G.L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44:98–106.

Hill, C.B., Y. Li, and G.L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46:1601–1605. Doi:10.2135/cropsci2005.11-0421

Hill, C.B., Y. Li, and G.L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci. 46:1606–1608.

Hill, C.B., K. Kim, L. Crull, B. W. Diers, and G. L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci. 49:1193–1200.

Hill, C.B., A. Chirumamilla, and G.L. Hartman. 2012. Resistance and virulence in the soybean-*Aphis glycines* interaction. Euphytica 186:635–646.

Hodgson, E. W., R. C. Venette, M. Abrahamson, and D. W. Ragsdale. 2005. Alate production of soybean aphid (Homoptera: Aphididae) in Minnesota. Environ. Entomol. 34: 1456-1463.

Hodgson, E.W, B.P. McCornack, K. Tilmon, and J.J. Knodel. 2012. Management recommendations for soybean aphid (Hemiptera: Aphididae) in the United States. J. Integ. Pest Mngmt. 3:1.

Hollingsworth, R. G., D. C. Steinkraus, and N. P. Tugwell. 1997. Responses of Arkansas populations of tarnished plant bugs (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. 90: 21-26.

Hopkins R. J., A. N. E. Birch, D. W. Griffiths, R. Baur, E. Städle, and R. G. Mckinlay. 1997. Leaf surface compounds and oviposition preference of turnip root fly *Delia floralis*: The role of glucosinolate and nonglucosinolate compounds. J. Chem. Ecol. 23:629–643.

Hoy, M. A. 1998. Myths: models and mitigation of resistance to pesticides. Phil. Trans. R. Soc. B 353: 1787–1795.

Hurley, T. and P. Mitchell. 2017. Value of neonicotinoid seed treatments to US soybean farmers. Pest Manag. Sci. 73: 102-112.

Husson, F., J. Josse, S. Le, and J. Mazet. 2016. FactoMineR: Multivariate Exploratory Data Analysis and Data Mining. R package version 1.32.

Iquira, E., S. Humira, and B. François. 2015. Association mapping of qtls for sclerotinia stem rot resistance in a collection of soybean plant introductions using a genotyping by sequencing (GBS) approach. BMC Plant Biol. 15: 5.

IRAC (Insecticide Resistance Action Committee). 2016. IRAC Susceptibility Test Method 019, Version 3.4. www.irac-online.org/methods/aphids-adultnymphs/

Jin, L., H. Zhang, Y. Lu, Y. Yang, K. Wu, B. E. Tabashnik, and Y. Wu. 2015. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. Nature Biotech. 33: 169-174.

Johnson, K. D., M. E. O'Neal, D. W. Ragsdale, and C. D. DiFonzo. 2009. Probability of cost-effective management of soybean aphid (Hemiptera: Aphididae) in North America. J. Econ. Entomol. 102: 2101-2108.

J**un, T. H., M. A. Rouf Mian, and A. P. Michel. 2012.** Genetic Mapping Revealed Two Loci for Soybean Aphid Resistance in PI 567301B. Theor. Appl. Genet. 124: 13-22.

Kaiser, M. E., T. Noma, M. J. Brewer, K. S., J. R. Vockeroth, & S. D. Gaimari. 2007. Hymenopteran parasitoids and dipteran predators found using soybean aphid after its 123aculate123n United States invasion. Ann. Entomol. Soc. Am. 100: 196-205.

Kandel, D. R., Tilmon, K. J., & Shuster, T. L. 2015. Effect of host plant resistance and seed treatments on soybean aphids (Hemiptera: Aphididae) and their natural enemies. J Entomol. Sci. 50: 186-205.

Kaser, J. M. 2016. Risk and efficacy in biological control: an evaluation of the aphid parasitoid *Aphelinus certus* in North America. Doctoral dissertation, University of Minnesota.

Kea, W. C., S. G. Turnipseed, G. R. Carner. 1978. Influence of resistant soybeans on the susceptibility of lepidopterous pests to insecticides. J.Econ. Entomol. 71: 58–60.

Kieckhefer, R. W., J. L. Gellner, and W. E. Riedell. 1995. Evaluation of the aphid-day standard as a predictor of yield loss caused by cereal aphids. Agron. J. 87:785-788.

Kim, C., G. Schaible, L. Garrett, R. Lubowski, and D. Lee. 2008a. Economic impacts of the US soybean aphid infestation: A multi-regional competitive dynamic analysis. Agric. Econ. Res. 37:227–242.

Kim, K., C. B. Hill, G. L. Hartman, M. A. R. Mian, and B. W. Diers. 2008b. Discovery of soybean aphid biotypes. Crop Sci. 48:923-928.

Kim, K. S., S. Bellendir, K. A. Hudson, C. B. Hill, G. L. Hartman, D.L. Hyten, M. E. Hudson, and B. W. Diers. 2010a. Fine mapping the soybean aphid resistance gene Rag1 in soybean. Theor. Appl. Genet. 120: 1063-1071.

Kim, K. S., C. B. Hill, G. L. Hartman, D. L. Hyten, M. E. Hudson, and B. W. Diers. 2010b. Fine Mapping of the Soybean Aphid-Resistance Gene Rag2 in Soybean PI 200538. Theor. Appl. Genet. 121: 599-610.

Kliot, A. and M. Ghanim 2012. Fitness costs associated with insecticide resistance. Pest. Manag. Sci. 68: 1431–1437.

Koch K. A., B. D. Potter, D. W. Ragsdale. 2010. Non-target impacts of soybean rust fungicides on the fungal entomopathogens of soybean aphid. J. Invertebr. Pathol. 103: 156-64.

Koch, R. L., & Costamagna, A. C. 2017. Reaping benefits from an invasive species: role of Harmonia axyridis in natural biological control of Aphis glycines in North America. BioControl, 62(3), 331-340.

Koch, R. L., B. D. Potter, P. A. Glogoza, E. W. Hodgson, C. H. Krupke, J. F. Tooker, C. D. DiFonzo, A. P. Michel, K. J. Tilmon, T. J. Prochaska, J. J. Knodel, R. J. Wright, T. E. Hunt, B. Jensen, A. J. Varenhorst, B. P. McCornack, K. A. Estes, and J. L. Spencer. 2016. Biology and economics of recommendations for insecticide-based management of soybean aphid. Plant Health Prog. 17:265-269.

Komatsu, K., M. Takahashi, and Y. Nakazawa. 2008. Antibiosis resistance of QTL introgressive soybean lines to common cutworm (*Spodoptera litura* Fabricius). Crop Sci. 48:527–532.

Kraiss, H., and E.M. Cullen. 2008. Efficacy and nontarget effects of reduced-risk insecticides on *Aphis glycines* (Hemiptera: Aphididae) and its biological control agent *Harmonia*
axyridis. J. Econ. Entomol. 101:391-398.

Krupke, C. H., A. M Alford, E. M. Cullen, E. W. Hodgson, J. J. Knodel, B. McCornack, B. D. Potter, M. I. Spigler, K. Tilmon, and K. Welch. 2017. Assessing the value and pest management window provided by neonicotinoid seed treatments for management of soybean aphid (*Aphis glycines* Matsumura) in the Upper Midwestern United States. Pest. Manag. Sci. 64.

Kumar, B., A. Talukdar, I. Bala, K. Verma, S. K. Lal, R. L. Sapra, B. Namita, S. Chander, and R. Tiwari. 2014. Population structure and association mapping studies for important agronomic traits in soybean J. Genet. 93: 775-784.

Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: An R Package for multivariate analysis. J. Stat. Softw. 25: 1-18.

Lettre G. 2011. Recent progress in the study of the genetics of height. Hum. Genet. 129: 465–472.

Li, Y. C.B. Hill, and G. L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97:1106-1111.

Li, Y., Zou, J., M. Li, D. D. Bilgin, L. O. Vodkin, G. L. Hartman and S. J. Clough. 2008. Soybean defense responses to the soybean aphid. New Phytol. 179: 185-195

Li, N. 2012. Pyrethroid resistance in insects: genes, mechanisms, and regulation. Pp. 457-468 In F. Perveen (ed.), Insecticides – advances in integrated pest management, InTech, Rijeka, Croatia.

Liu G., H. Xing, Y. Diao, X. Yang, D. Sun, Q. Wang, N. Qi, H. Lin. 2015. Identification of resistance to soybean aphids in early germplasm. Crop Sci. 54: 2707-2712.

Liu, H., Z. Che, X. Zeng, G. Zhang, H. Wang, and D. Yu. 2016. Identification of single nucleotide polymorphisms in soybean associated with resistance to common cutworm (*Spodoptera litura* Fabricius). Euphytica 209: 49–62.

Lundgren, J. G., L. S. Hesler, K. Tilmon, K. Dashiell, R. Scott. 2009. Direct effects of soybean varietal selection and *Aphis glycines*-resistant soybeans on natural enemies. Arthropod-Plant Interactions. 3: 9-16.

Macedo, T.B., C.S. Bastos, L. G. Higley, K. R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid injury. J. Econ. Entomol. 96:188-193.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, K. Hornik, 2016. Cluster: Cluster Analysis Basics and Extensions. R package version 2.0.5.

Magalhaes, L. C., T. E. Hunt, and B. D. Siegfried. 2008. Development of methods to evaluate susceptibility of soybean aphid to imidacloprid and thiamethoxam at lethal and sublethal concentrations. Entomol. Exp. Appl. 128: 330–336.

Marchi-Werle, I., T.M. Heng-Moss, T.E. Hunt, E.L.L. Baldin, and L. M. Baird. 2014 Characterization of peroxidase changes in tolerant and susceptible soybeans challenged by soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 107:1985-1991.

Marchi-Werle, L., R. R. Pereira, J. C. Reese, T. M. Heng-Moss, and T. E. Hunt. 2017. Yield response of tolerant and susceptible soybean to the soybean aphid. Agron. J. 109: 1663-1669.

Martin, L. J., G. Gao, G. Kang. Y. Fang, and J. G. Woo. 2009. Improving the signal-to-noise ratio in genome-wide association studies. Genet. Epidemiol. 33: S29–S32.

Mascarenhas, R. N. and D. J. Boethel. 2000. Development of diagnostic concentrations for insecticide resistance monitoring in soybean looper (Lepidoptera: Noctuidae) larvae using an artificial diet overlay bioassay. J. Econ. Entomol. 93: 897-904.

Maxwell, F. G. and R. H. Painter. 1962. Auxin content of extracts of certain tolerant and susceptible host plants of *Toxoptera graminum* (Rond.), *Macrosiphum pisi* (Harr.) and *Therioaphis 125aculate* (Buck.) and relation to host plant resistance. Jour. Econ. Ent. 55(l):46-56.

McCarville, M.T., E.W. Hodgson, and M.E. O'Neal. 2012. Soybean aphid-resistant soybean varieties for Iowa. Agriculture and Environment Extension Publications.

McCarville, M. T. and M. E. O'Neal; 2013. Soybean aphid (Aphididae: Hemiptera) population growth as affected by host plant resistance and an insecticidal seed treatment. J. Econ. Entomol.106: 1302–1309.

McCarville, M. T., D. H. Soh, G. L. Tylka, and M. E. O'Neal. 2014a. Above ground feeding by soybean aphid, *Aphis glycines,* affects soybean cyst nematode, *Heterodera glycines*, reproduction belowground. PloS ONE 9:e86415.

McCarville, M.T., M.E. O'Neil, B.D. Potter, K.J. Tilmon, E.M. Cullen, B.P. McCornack, J.F. Tooker, and D.A. Prichsmann-Voldseth. 2014b. One gene versus two: A regional study on the efficacy of single gene versus pyramided resistance for soybean aphid management. J. Econ. Entomol. 107:1680-1687.

McCornack, B.P., D.W. Ragsdale, and R.C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. J. Econ. Entomol. 97: 854-861.

Mensah, C., C. DiFonzo, R. L. Nelson, and D. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45:2228–2233. Michel A.P., O. Mittapalli, and M.A. Rouf Mian. 2011. Evolution of soybean aphid biotypes: understanding and managing virulence to host-plant resistance, Soybean – molecular aspects of breeding. A. Sudaric (Ed.), ISBN: 978-953-307-240-1, InTech,

Michel, A. P., W. Zhang, J. K. Jung, S. Kang, and M. A. Rouf Mian. 2009. Population genetic structure of *Aphis glycines* Environ. Entomol. 38: 1301-1311.

Miller, A. L. E., K. Tindall, and B. R. Leonard. 2010. Bioassays for monitoring insecticide resistance. J. Vis. Exp. 46: 2129.

Mitchell, P. D. and W. D. Hutchison. 2009. Decision making and economic risk in IPM. In: Radcliffe, E. B., Hutchison, W. D., & Cancelado, R. E. (Eds.). Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press.

Moharramipour, S., H. Tsumuki, K. Sato, S. Murata, and K. Kanehisa. 1997. Effects of leaf color, epicuticular wax amount and gramine content in barley hybrids on cereal aphid populations. Appl. Entomol. Zool. 32:1–8.

Morawo T., H. Fadamiro. 2014. Attraction of two larval parasitoids with varying degree of host specificity to single components and a binary mixture of host-related plant volatiles. Chemecology 24: 127-135.

Myles, S., J. Peiffer, P. J .Brown, E. S. Ersoz, Z. Zhang, D. E. Costich, and E. S. Buckler. 2009. Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21:2194–2202.

Naranjo, S. E., Ellsworth, P. C., & Frisvold, G. B. 2015. Economic value of biological control in integrated pest management of managed plant systems. Annu. Rev. Entomol. 60: 621-645.

NASS. 2015. United States Department of Agriculture: National Agriculture Statistics Service. http://www.nass.usda.gov/

Nielson, M. W., and R. O. Kuehl. 1982. Screening efficacy of spotted alfalfa aphid 1 biotypes and genic systems for resistance in alfalfa. Environ. Entomol. 11: 989-996.

Orantes L. C., W. Zhang, M. A. R. Mian, and A. P. Michel. 2012. Maintaining genetic diversity and population panmixia through dispersal and not gene flow in a holocyclic heteroecious aphid species. Heredity 109: 127-134.

Painter, R. H. 1951. Insect resistance in crop plants. The Macmillan Company, New York.

Painter, R. H. 1958. Resistance of plants to insects. Annu. Rev. Entomol. 3: 267-290.

Paradis E., J. Claude and K. Strimmer 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289-290.

Park J. H., M. H. Gail. C. R. Weinberg, R. J. Carroll, C. C. Chung, Z. Wang, S. J. Chanock, J. F. Fraumeni, N. Chatterjee. 2011. Distribution of allele frequencies and effect sizes and their interrelationships for common genetic susceptibility variants. Proc. Natl Acad. Sci. 108: 18026–18031.

Pedigo, L.P. 1995. Closing the gap between IPM theory and practice. J. Agric. Entomol. 12:171-181.

Pedigo, L. P. and M. E. Rice. 2009. Entomology and pest management. 6th ed. Pearson Prentice Hall, Inc. Upper Saddle River, NJ.

Pezzini, D. T. and R. L. Koch. 2015. Compatibility of flonicamid and a formulated mixture of pyrethrins and azadirachtin with predators for soybean aphid (Hemiptera: Aphididae) management. Biocontrol Sci. Technol. 25: 1024-1035.

Pierson, L.M., T.M. Heng-Moss, T.E. Hunt, and J.C Reese. 2010. Categorizing the resistance of soybean genotypes to the soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 103:1405-1411.

Pillemer, E. A.and W. M. Tingey. 1976. Hooked trichomes: a physical plant barrier to a major agricultural pest. Science 193: 482–484.

Prabhaker, N., N. C. Toscano, T. J. Henneberry, S. J. Castle, D. Weddle. 1996. Assessment of two bioassay techniques for resistance monitoring of silverleaf whitefly (Homoptera: Aleyrodidae) in California. J. Econ. Entomol. 89: 805-815.

Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genet. 38: 904-909.

Prochaska, T.J., L.M Pierson, E.L.L. Baldin, T.E. Hunt, T.M. Heng-Moss, and J.C. Reese. 2013. Evaluation of late vegetative and reproductive stage soybeans for

resistance to soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 106:1036-1044.

Qin, J., A. Shi, B. Mou., G. Bhattarai, W. Yang, Y. Weng, and D. Motes. 2017. Association mapping of aphid resistance in USDA cowpea (*Vigna unguiculata* L. Walp.) core collection using SNPs. Euphytica, 213: 36.

Qu, Y., D. Xiao, J. Li, Z. Chen, A. Biondi, N. Desneux, X. Gao, and D. Song. 2015. Sublethal and hormesis effects of imidacloprid on the soybean aphid *Aphis glycines*. Ecotoxicol. 24: 479-87.

Qu, Y., D. Xiao, J. Liu, Z. Chen, L. Song, N. Desneux, G. Benelli, X. Gao, D. Song. 2017. Sublethal and hormesis effects of beta-cypermethrin on the biology, life table parameters and reproductive potential of soybean aphid *Aphis glycines*. Ecotoxicology. In press.

Quisenberry, S. S. and D. J. Schotzko, D. J. 1994. Integration of plant resistance with pest management methods in crop production systems. J. Agric. Entomol, 11: 279-290.

Radcliffe, E. B., Hutchison, W. D., & Cancelado, R. E. (Eds.). 2009. Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press.

Ragsdale, D.W., D.J. Voegtlin, R.J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97:204-208.

Ragsdale D. W., B. P. McCornack, R.C. Venette, B. D. Potter, I. V. MacRae, E. W. Hodgson, M. E. O'Neal, K. D. Johnson, R. J. O'Neil, C. D. DiFonzo, T. E. Hunt, P. A. Glogoza, and E. M. Cullen. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 100: 1258-1267.

Ragsdale, D. W., D. A. Landis, J. Brodeur, G.E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annu. Rev. Entomol. 56: 375-399.

Rambaut A. 2012. Figtree v1.4.0, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software.figtreetree.

Ribeiro, M. G. P. M. 2017. Baseline susceptibility, resistance detection and selection for resistance in *Aphis glycines* (Hemiptera: Aphididae) to the neonicotinoid insecticide, thiamethoxam. Ph.D. dissertation, University of Nebraska, Lincoln.

Richardson, M. 2012. Temperature influences the expression of resistance of soybean (*Glycine max*) to the soybean aphid (*Aphis glycines*). J. Appl. Entomol. 136:641-645.

Rogers, R. R., W. A. Russell, and J. C. Owens. 1976. Evaluation of a vertical-pull technique in population improvement of maize for corn rootworm tolerance. Crop Sci. 16:591–594.

Rosenheim, J. A. and M. A. Hoy. 1989. Confidence intervals for the Abbott's formula correction of bioassay data for control response. J. Econ. Entomol. 82: 331–335.

Rouf Mian, M. A., R. B. Hammond, and S. K. St. Martin. 2008. New plant introductions with resistance to the soybean aphid. Crop Sci. 48:1055–1061.

Ruppel, R. F. 1983. Cumulative insect-days as an index of crop protection J. Econ. Entomol. 76: 375-377.

Rutledge, C. E, R. J. O'Neil, T. B. Fox, D. A. Landis. 2004. Soybean aphid predators and their use in integrated pest management. Ann. Entomol. Soc. Am. 97:240–248.

Saitou, N., and M. Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

SAS Institute. 2017. The SAS system for windows: Release 9.4. SAS Institute, Inc., Cary, NC.

Schmidt, N. P., M. E. O'Neal, P. F. Anderson, D. Lagos, D. Voegtlin, W. Bailey, P. Caragea, E. Cullen, C. DiFonzo, K. Elliott, C. Gratton, D. Johnson, C. H. Krupke, B. McCornack, R. O'Neil, D. W. Ragsdale, K. J. Tilmon, and J. Whitworth. 2012. Spatial distribution of *Aphis glycines* (Hemiptera: Aphididae): a summary of the suction trap network. J. Econ. Entomol. 105: 259-271.

Schwantes-An, T. H., H. Sung, J. A. Sabourin, C. M. Justice. A. J. Sorant, and A. F. 2016. Type I error rates of rare single nucleotide variants are inflated in tests of association with non–normally distributed traits using simple linear regression methods. BMC Proc. 10: 62.

Scott, J. G., N. Liu, and Z. Wen. 1998. Insect cytochromes P450: diversity, insecticide resistance and tolerance to plant toxins. Comp. Biochem. Physiol. Part D Genomics Proteomics 121: 147-155.

Shade, R. E., M. J. Doskocil, and N. P. Maxon. 1979. Potato leafhopper resistance in glandular-haired alfalfa species. Crop Sci. 19:287–289.

Shockley, F. W., E. A. Backus, M. R. Ellersieck, D. W. Johnson, and M. McCaslin 2002. Glandular-haired alfalfa resistance to potato leafhopper (Homoptera: Cicadellidae) and hopperburn: development of resistance indices. J. Econ. Entomol. 95: 437-447.

Stoner, K. A. 1990. Glossy leaf wax and host-plant resistance to insects in Brassica oleracea L. under natural infestations. Environ. Entomol. 19:730–739.

Singh, K. K. A. Tomar, and H. S. Rathore. 2000. Scope and limitations of neem products and other botanicals in plant protection: a perspective. P. 70. In L. M. L Nollet and H. S. Rathore (ed.) Handbook of pesticides: methods of pesticide residue analysis. CRC Press, Boca Raton, FL.

Smith, C.M. 2005. Plant resistance to arthropods: molecular and conventional approaches. Springer. Dordrecht, The Netherlands.

Smith, C. M., and Chuang, W. P. 2014. Plant resistance to aphid feeding: behavioral, physiological, genetic and molecular cues regulate aphid host selection and feeding. Pest Manag. Sci. 70: 528-540.

Snodgrass, G.L. 1996. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). J. Econ. Entomol. 89: 1053-1059.

Song, F., S. M. Swinton, C. DiFonzo, M. O'Neal, and D.W. Ragsdale. 2006. Profitability analysis of soybean aphid control treatments in three north-central states. Staff Paper No. 2006–24. Michigan State Univ., Dep. Agric. Economics, East Lansing, MI.

Song, F. and S. M. Swinton. 2009. Returns to integrated pest management research and outreach for soybean aphid. J. Econ. Entomol. 102:2116–2125.

Song, Q., D. L. Hyten, G. Jia, C. V. Quigley, E. W. Fickus, R. L. Nelson, P. B. Cregan. 2013. Development and evaluation of SoySNP50K, a high-density genotyping array for soybean. PloS ONE 8(1): e54985.

Song, Q., D. L. Hyten, G. Jia, C. V. Quigley, E. W. Fickus, R. L. Nelson, P. B. Cregan. 2015. Fingerprinting soybean germplasm and its utility in genomic research. G3. 5: 1999-2006.

Sparks, T. C. and R. Nauen. 2016. IRAC: Mode of action classification and insecticide resistance management, Pest. Biochem. Physiol. 121: 122-128.

Spencer, C. C., Z. Su, P. Donnelly, J. Marchini, J. 2009. Designing genome-wide association studies: sample size, power, imputation, and the choice of genotyping chip. PloS genetics, 5(5), e1000477.

Stern, V. M. R. F., Smith, R., Van den Bosch, R., & Hagen, K. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. *Calif. Agri.*, 29: 81-101.

Stout, M., and J. Davis. 2009. Keys to the increased use of host plant resistance in integrated pest management. In Integrated Pest Management: Innovation-Development Process (pp. 163-181). Springer Netherlands.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14:179–185.

Tabadkani, S. M., M. Khoobdel, and H. R. Tavakoli. 2017. Host resistance enhances susceptibility of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) to herbal extract of *Echinophora platyloba*. Entomol. Res. 47: 28-34.

Tabangin, M. E., J. G. Woo, and L. J. Martin. 2009. The effect of minor allele frequency on the likelihood of obtaining false positives. BMC Proc. 3: S41.

Tabashnik B. E. 1990. Modeling and evaluation of resistance management tactics. Pp. 153–182 In R.T. Roush and B. E. Tabashnik (eds). Pesticide resistance in arthropods. Chapman and Hall, New York, NY.

Tabashnik, B. E., J. B. J. Van Rensburg, and Y. Carriere. 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. J. Econ. Entomol. 102:2011:2025.

Tabashnik, B. E., D. Mota-Sanchez, M. E. Whalon, R. M. Hollingworth, and Y. Carriere. 2014. Defining terms for proactive management of resistance to Bt crops and pesticides. J. Econ. Entomol. 107: 496-507.

Tanksley, S. D., & S. R. McCouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1066.

Tilmon, K. J., E. W. Hodgson, M. E. O'Neal, and D. W. Ragsdale. 2011. Biology of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. J. Integ. Pest Mngmt. 2:2.

Tran, A. K., T. M. Alves, and R. L. Koch. 2016. Potential for sulfoxaflor to improve conservation biological control of *Aphis glycines* (Hemiptera: Aphididae) in soybean. J. Econ. Entomol. 109: 2105-2114.

USDA, ARS. 2015. National Genetic Resources Program. Germplasm Resources Information Network – (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov.4/cgi-bin/npgs/html/desc.pl?51111 (last accessed 4 May 2015)

USDA-ARS. 2016. Descriptors for soybean. National Germplasm Resources Laboratory. https://npgsweb.ars-grin.gov/gringlobal/cropdetail.aspx?type=descriptor&id=51 (accessed 27 Jun. 2016)

USDA-NASS. 2016. National Statistics for Soybeans. USDA-NASS. https://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS

Van Der Plank, J.E. 1966. Horizontal (polygenic) and vertical (oligogenic) resistance against blight. Am. Potato J. 43: 43.

Varenhorst, A. J., M. T. McCarville, M. E. O'Neal. 2015a. Reduced fitness of virulent *Aphis glycines* (Hemiptera: Aphididae) biotypes may influence the longevity of resistance genes in soybean. PloS ONE 10(9): e0138252.

Varenhorst, A. J., M. T. McCarville, M. E. O'Neal. 2015b. An induced susceptibility response in soybean promotes avirulent *Aphis glycines* (Hemiptera: Aphididae) populations on resistant soybean, Environ. Entomol. 44: 658–667.

Varenhorst, A. J., M. T. McCarville, M. E. O'Neal. 2015. Determining the duration of *Aphis glycines* (Hemiptera: Aphididae) induced susceptibility effect in soybean. Arthropod Plant Interact. 9: 457-464.

Venette, R. C., and D. W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97: 219-226.

Visser, J. H. 1986. Host odor perception in phytophagous insects. Ann. Rev. Entomol. 31:121–44.

Vuong, T. D., H. Sonah, C. G. Meinhardt, R. Deshmukh, S. Kadam, R. L. Nelson, J. G. Shannon, and H. T. Nguyen. 2015. Genetic architecture of cyst nematode resistance revealed by genome-wide association study in soybean. BMC Genomics 16: 593.

Wahlroos, V., and A. I. Virtanen. 1959. The precursors of 6 MBOA in maize and wheat plants, their isolations and some of their properties. Acta Chem. Scan. 13:1906–1908.

Waiss, A. C., B. G. Chan, C. A. Elliger, B. R. Wiseman, W. W. McMillian, N. W. Widstrom, M. S. Zuber, and A. J. Keaster. 1979. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. J. Econ. Entomol. 72: 256-258.

Wang Q., W. Xu, and S. C. Yan. 2011. Research on insecticide resistance of *Aphis glycines* in Heilongjiang Province. Dongbei Nongye Daxue Xuebao 42: 137-140.

Wang, N., J. M. Akey, K. Zhang, R. Chakraborty, and L. Jin. 2002. Distribution of recombination crossovers and the origin of haplotype blocks: The interplay of population history, recombination, and mutation. Am. J. Hum. Genet. 71:1227–1234.

Wang, Q., S. Yan, W. Xu, and C. Wang. 2012. Research on resistance of soybean aphid (*Aphis glycines*) in Heilongjiang Province. Agric. Sci. Bull. 28: 218–221.

Wang, H., H. Yan, H. Du, M. Chao, Z. Gao, and D. Yu. 2015. Mapping quantitative trait loci associated with soybean resistance to common cutworm and soybean compensatory growth after defoliation using SNP marker-based genome- wide association analysis. Mol. Breed. 35:168.

Weinzierl, R.A. 2009. Integrating pesticides with biotic and biological control for arthropod pest management. In: E. B. Radcliffe, W. D. Hutchison & R. E.

Cancelado [eds.], Radcliffe's IPM World Textbook, URL: https://ipmworld.umn.edu, University of Minnesota, St. Paul, MN.

Wenger, J., M. Ramstad, M.A. Rouf Mian, and A. Michel. 2014. The use of refuge in host plant resistance systems for the control of virulent biotype adaptation in the soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 107:1599-1609.

Whalen, R. and J.P. Harmon. 2015. Temperature alters the interaction between a herbivore and a resistant host plant. Arth.-Plant Int. 3: 233-240.

Wiarda, S.L., W.R. Fehr, and M.E. O'Neal. 2012. Soybean aphid (Hemiptera: Aphididae) development on soybean with *Rag1* alone, *Rag2* alone, and both genes combined. J. Econ. Entomol. 105:252-258.

Willrich, M. M., B. Rogers Leonard, D. R. Cook. 2003. Laboratory and field evaluations of insecticide toxicity to stink bugs (Heteroptera: Pentatomidae). J. Cotton Sci. 7: 156-163.

Wiseman, B. R. 1985. Types and mechanisms of host plant resistance to insect attack. Int. J. Trop. Insect. Sci. 6: 239-242.

Xi, J., Y. Pan, R. Bi, X. Gao, X. Chen, T. Peng, M. Zhang, H. Zhang, X. Hu, and Q. Shang. 2015. Elevated expression of esterase and cytochrome P450 are related with lambda-cyhalothrin resistance and lead to cross resistance in *Aphis glycines* Matsumura. Pestic. Biochem. Physiol. 118: 77-81. Doi: 10.1016/j.pestbp.2014.12.002

Xiao, L., Y. Hu, B. Wang, and T. Wu. 2013. Genetic Mapping of a Novel Gene for Soybean Aphid Resistance in Soybean (Glycine Max [L.] Merr.) Line P203 from China. Theor. Appl. Genet. 126 (9): 2279-2287.

Young, F. L., A. G. Ogg, R. I. Papendick, D. C. Thill, J. R. Alldredge. 1994. Tillage and weed management affects winter wheat yield in an integrated pest management system. Agron. J. 86: 147-154.

Zhang, G., C. Gu, and D. Wang. 2009. Molecular mapping of soybean aphid resistance genesin PI 567541B. Theor. Appl. Genet.118: 473

Zhang, G., C. Gu, and D. Wang. 2010. A Novel Locus for Soybean Aphid Resistance. Theor. Appl. Genet.120: 1183-1191.

Zhang, G., C. Gu, and D. Wang. 2013. Mapping and validation of a gene for soybean aphid resistance in PI 567537. Mol. Breeding 32: 131-138.

Zhang, J., Q. Song, P. B. Cregan, R. L. Nelson, X. Wang, J. Wu and G. L. Jiang. 2015a. Genome-wide association study for flowering time, maturity dates and plant height in early maturing soybean (*Glycine max*) germplasm. BMC genomics 16: 217

Zhang, J., A. Singh, D.S. Mueller and A.K. Singh. 2015b. Genome-wide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. Plant J. 84: 1124-1136.

Zhang, J., Q. Song, P. B. Cregan and G. L. Jiang. 2016. Genome-wide association study, genomic prediction and marker-assisted selection for seed weight in soybean (*Glycine max*). Theor. Appl. Genet. 129: 117-130.

Appendix 1

Inheritance of soybean aphid resistance in PI 639537

Relatively few varieties containing soybean aphid resistance genes are available for growers (Hanson et al. 2016a). The inheritance of soybean aphid resistance (e.g., dominant or recessive) in PI 639537, a soybean line identified as aphid-resistant in Chapter 1 (Hanson et al. 2016b), was determined by crossing PI 639537 with M05-363022, a high-yielding but aphid-susceptible line from the University of Minnesota soybean breeding program.

Parent plants were grown in 30-cm diameter by 25-cm height pots filled to a height of 19.5 cm with potting soil (Sunshine LC8, Sun Gro Horticulture Products, Agawam, MA) and maintained in an environmental growth chamber at 25°C, 16:8h photoperiod. Seeds were planted at a depth of 2 cm and a 1-cm layer of sand was added to the top of the soil to minimize fungus gnat infestation. Flowering was synchronized by planting PI 639567 ten days earlier than M05-363022. PI 639567 male × M05-363022 female crosses were performed.

F1 progeny were grown under greenhouse conditions (i.e., 25°C, 16:8h photoperiod, and approximately 65% RH) initially in small 10-cm^3 pots and then transferred to the larger pot sizes used for parental lines explained previously to grow plants to maturity. Successful crosses were differentiated by a black hilum on F1-produced seed, which is dominant over the white hilum of M05-363022 (Hernandez-Garcia 2013). The F2 generation was grown in a similar manner as the F1 generation.

At early V2 growth stage (Fehr and Caviness 1977), two apterous adult aphids were placed in a clip cage on the abaxial side of first fully expanded trifolioate leaflet for each F2 plant and parental checks (Hanson et al. 2016b). Surviving adult aphids and nymphs produced were recorded 24 and 48 h after infestation.

During preliminary experiments with parental lines and in parental lines during the F2 screening, net aphid population growth per day was consistently less than 1 aphid per day on resistant PI 639537, and greater than 1 on susceptible M05-363022. This differentiation was used to categorize plants as susceptible versus resistant. PROC FREQ

in SAS 9.4 was used to test whether resistance ratings significantly differed from a 3:1 segregation ratio (i.e., 75% resistant and 25% susceptible or vice versa).

The ratio of susceptible and resistant F2 plants was not significantly different from a 3:1 resistance:susceptibility ratio ($\chi^2 = 0286$, $df = 1$, $p = 0.593$), which indicated that resistance in PI 639537 is inherited as a dominant trait.

Resistance from PI 639537 should be easily introgressed into elite soybean lines as resistant plants were easily distinguished in the clip cage assays. The F2 population produced here could also be used for additional breeding and mapping experiments to determine the location of the causal resistance gene for PI 639537.

Table 1. Expected and observed proportions of resistance ratings and mean change in aphid counts per day.

Rating	*n*	Expected	Observed	± 95% C.L.		Mean daily aphid growth	± 95% C.L.	
Susceptible	12	0.25	0.286	0.170	0.439	3.067	1.096	5.037
Resistant	30	0.75	0.714	0.561	0.830	0.020	-0.162	0.202

Appendix 2

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